Supplementary Information

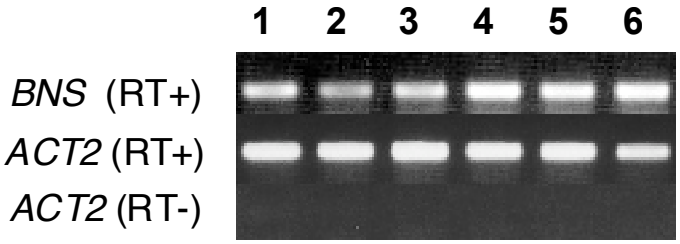
Bisulfite sequencing procedure

0.5 μg of genomic DNA isolated from rosette leaves was digested with *Hind*III and *Dra*I in a 20 μl reaction at 37 °C for more than 5 hours. The DNA was heated at 95 °C for 2 min, and cooled on ice, and then denatured by addition of 2.2 μl of 3M NaOH and incubation for 15 min at 37 °C. To prepare the urea/bisulfite solution, 7.5 g of Urea (Wako) and 7.6 g of sodium metabisulfite (MERK) was dissolved in 20 ml H_20 (pH 5.0 adjusted with 10M NaOH). In a 0.5 ml PCR tube, the denatured DNA, 208 μl of urea/bisulfite solution and 12 μl of 10mM hydroquinone (SIGMA) were mixed, and overlaid with mineral oil. The samples were subjected to 30 cycles of 95 °C for 30 sec, 55 °C for 15 min, followed by incubation at 55 °C for more than 15 hours in the PCR machine. The bisulfite treated DNA was purified using the GENE CLEAN kit (Q-BIOgene) and eluted with 20 μl of the supplied elution buffer, and desulfonation was performed by addition of 2.2 μl of 3M NaOH and incubation for 15 min at 37 °C. The DNA was precipitated with 7.26 μl of 10 M ammonium acetate, 75 μl of 100% ethanol under addition of 0.5 μl of tRNA (10 mg/ml) at -80 °C for 15min, followed by a 70% ethanol wash. The precipitate was dissolved in 20 μl of 0.1 X TE buffer and stored at -20 °C. PCR reactions were performed in 50 μl reaction mixtures using 5 μl of bisulfite treated DNA as a template, starting with 94 °C for 3min, followed by 32- 40 cycles of 94 °C for 15 sec, 50 °C for 30 sec, 72 °C for 1 min, and further incubated at 72 °C for 10min. Amplified fragments were gel-purified and cloned into pGEM-T easy vector (Promega) and sequenced. *BNS* and LINE sequences were amplified by using four pairs of primers (Y is C or T, and R is G or A): upstream of *BNS* region (meBNS RF1: 5'-AGY TTT TTT TAT AAG YTA GAG GTT TTT AGT-3' and meBNS RR1: 5'-ACC TAT ATA TRA ATA TCT AAA ACA ATC TAC-3'); middle of *BNS* region (meBNS RF3: 5'-TTG TTT TAG ATA TTY ATA TAT AGG TTA TTG-3' and meBNS RR3: 5'-TCT CCA TTA CTT TCC TTA TRA CAT TTC A-3'); downstream of *BNS* region (meBNS RF2: 5'-TTG AAA TGT YAT AAG GAA AGT AAT GGA GA-3' and meBNS RR5: 5'-TTA RCT AAC TAT RCT TTT CTT TTA CC-3'); LINE sequence (meBNS RF4: 5'-TTY ATA YYT TYT GTG ATA TAY GGY GTA AG-3' and meBNS RR6: 5'-CAR CRA CAA ATT CTT AAA ATT TAT RCC ACC-3').

Natural accessions examined in Figure 5B

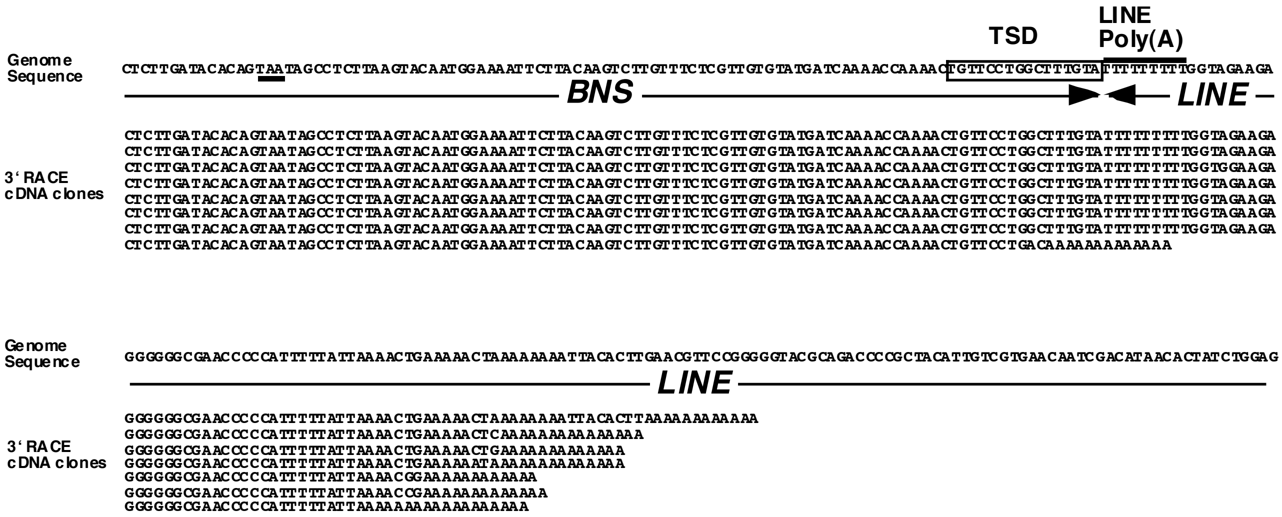
1, Sq-8; 2, CIBC-5; 3, CIBC-17; 4, Tamm-2; 5, Tamm-27; 6, Kz-1; 7, Kz-9; 8, Got-7; 9, Got-22; 10, Ren-1; 11, Ren-11; 12, Uod-1; 13, Uod-7; 14, Cvi-0; 15, Lz-0; 16, Ei-2; 17, Gu-0; 18, Ler-1; 19, Nd-1; 20, C24; 21, CS22491; 22, Wei-0; 23, Ws-0; 24, Yo-0; 25, Col-0; 26, An-1; 27, Van-0; 28, Br-0; 29, EST-1; 30, Ag-0; 31, Gy-0; 32, Ra-0; 33, Bay-0; 34, Ga-0; 35, Mrk-0; 36, Mz-0; 37, Wt-5; 38, Kas-1; 39, Ct-1; 40, Mr-0; 41, Tsu-1; 42, Mt-0; 43, Nok-3; 44, Wa-1; 45, Fei-0; 46, Se-0; 47, Ts-1; 48, Ts-5; 49, Pro-0; 50, LL-0; 51, Kondara; 52, Sakhdara; 53, Sordo; 54, Kin-0; 55, Ms-0; 56, Bur-0; 57, Edi-0; 58, Oy-0; 59, Ws-2; 60, RRS-7; 61, RRS-10; 62, Knox-10; 63, Knox-18; 64, Rmx-A02; 65, Rmx-A180; 66, Pna-17; 67, Pna-10; 68, Eden-1; 69, Eden-2; 70, Lov-1; 71, Lov-5; 72; Fab-2; 73, Fab-4; 74, Bil-5; 75, Bil-7; 76, Var2-1; 77, Var2-6; 78, Spr1-2; 79, Spr1-6; 80, Omo2-1; 81, Omo2-3; 82, Ull2-5; 83, Ull2-3; 84, Zdr-1; 85, Zdr-6; 86, Bor-1; 87, Bor-4; 88, Pu2-7; 89, Pu2-23; 90, Lp2-2; 91, Lp2-6; 92, HR-5; 93; HR-10; 94, NFA-8; 95, NFA-10; 96, Sq-1.

Supplementary Figure 1



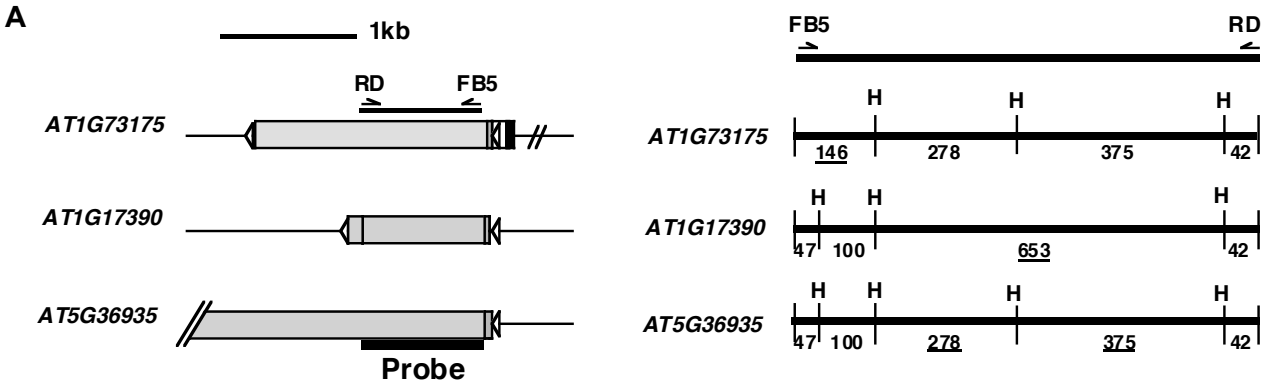
Expression of *BNS* mRNA was analyzed by RT-PCR in various wild-type tissues. Lanes 1-6 are root, leaf, stem, buds, flower, and seeds, respectively. *Actin2* (ACT2) with and without reverse transcriptase were used as controls.

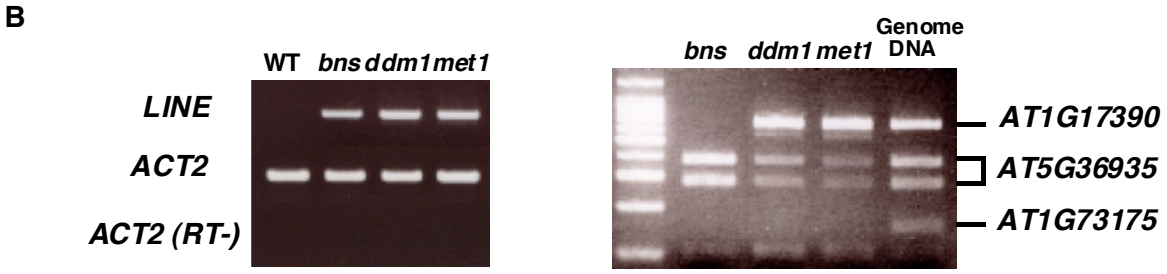
Supplementary Figure 2

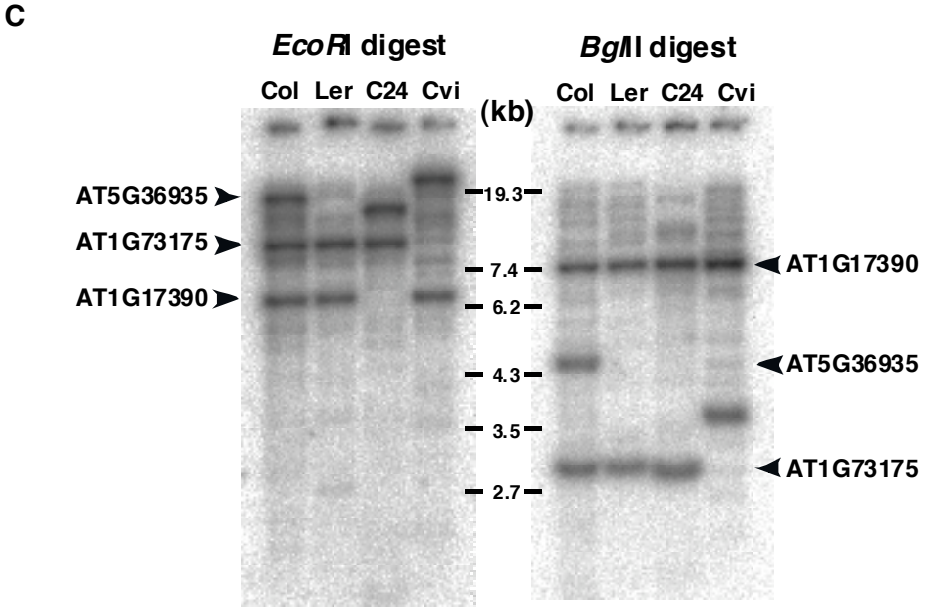


3' RACE of *BNS* transcripts in WT Col. RACE was performed as described in Materials and methods. Eight independent clones were sequenced, and aligned. TSD: target site duplication of the LINE insertion.

Supplementary Figure 3







Legend for Supplementary Figure 3

Expression of the LINE family in *ddm1*, *met1* and *bns*. (A) Left: a schematic diagram of the LINE family. The position of the primer pair used for RT-PCR is indicated. Note that the primers can amplify transcripts derived from the three LINE templates. Right: a restriction enzyme map of the RT-PCR fragments after *Hpa*II digestion. H; *Hpa*II sites. The numbers indicate the sizes of fragments in base-pair. (B) Left: RT-PCR for the LINE transcripts in *ddm1*, *met1* and *bns*. *Actin2* was used as a control. Right: The LINE transcripts amplified by the RT-PCR was gel-purified, and digested with *Hpa*II, and the reaction was separated on gel-electrophoresis. The origin of the fragments is indicated in the right of the picture. Interestingly, transcript was not detected in the LINE copy flanking the *BNS* gene (ATG73175). That might be because the LINE transcript in this locus was cleaved by RNAi machinery more efficiently than those in the other LINE copies. (C) Southern analysis for the LINE family. Genomic DNA from the four ecotypes was digested with *Eco*RI or *Bgl*II. The blots were hybridized with the probe indicated in A. Gene ID with arrowhead indicates hybridized band corresponding to the LINE sequences in the Col genome.