

| Footprint sequence* | Derivative | Female gametes | Male gametes |
|-------------------------------------|--------------------|----------------|--------------|
| CATCCGACGCTAGCGGC/CTAGCGGCTAGCCTAG | <i>bz-m39 (Ac)</i> | | |
| CATCCGACGCTAGCGGG/CTAGCGGCTAGCCTAG | <i>Bz'</i> | 15 | 19 |
| CATCCGACGCTAGCGGG/-TAGCGGCTAGCCTAG | <i>Bz'</i> | 6 | 14 |
| CATCCGACGCTAGCGG--/TAGCGGCTAGCCTAG | <i>Bz'</i> | | 5 |
| CATCCGACGCTAGCGGGC/--AGCGGCTAGCCTAG | <i>Bz'</i> | 1 | 4 |
| CATCCGACGCTAGCGGGC/CTAGCGGCTAGCCTAG | <i>Bz'</i> | | 2 |
| CATCCGACGCTAGCGG-/AGTAGCGGCTAGCCTAG | <i>Bz'</i> | 1 | 2 |
| CATCCGACGCTAGCGGG/--AGCGGCTAGCCTAG | <i>Bz'</i> | | 2 |
| CATCCGACGCTAGCG--/-TAGCGGCTAGCCTAG | <i>Bz'</i> | | 2 |
| CATCCGACGCTAGCGGGG/CTAGCGGCTAGCCTAG | <i>Bz'</i> | 1 | 1 |
| CATCCGACGCTAGCGGC/-----TAGCCTAG | <i>Bz'</i> | | 1 |
| CATCCGACGCTAGCGG--/--AGCGGCTAGCCTAG | <i>Bz'</i> | | 1 |
| CATCCGACGCTAGCGGC/-----GGCTAGCCTAG | <i>Bz'</i> | 1 | |
| | | 25 | 53 |

* TSD and footprint sequences in red

Supplemental Figure 1

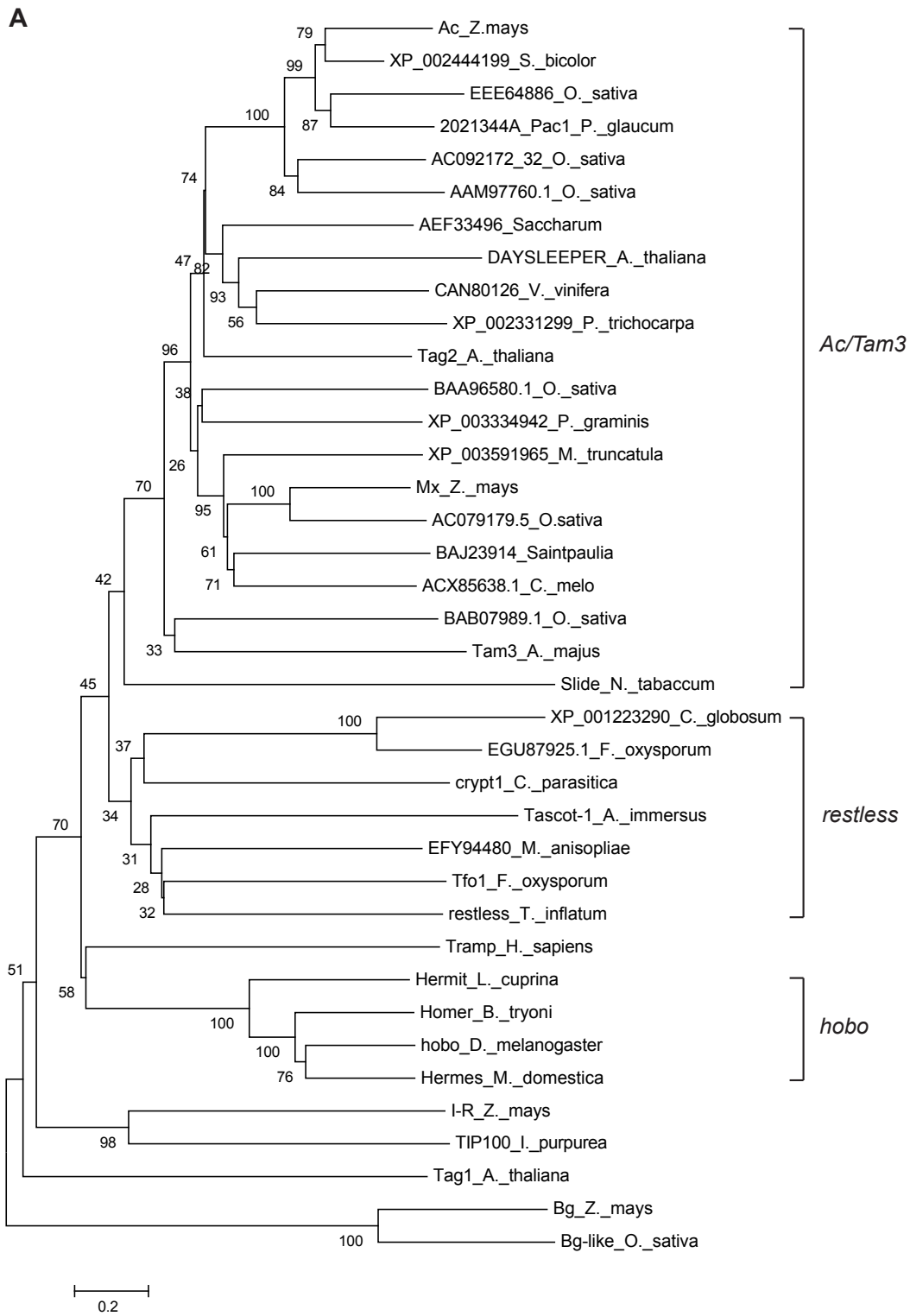
Supplemental Figure 1. Excision footprints among *Bz'* revertants recovered from female and male gametes



Supplemental Figure 2

Supplemental Figure 2. *bz-m39.31* phenotype.

Finely spotted kernels from a cross between *wx-m7(Ac)* as male and *bz-m39.31* as female. The *Ds39.31* element shows minimal somatic excision activity. Some of the fine spots produced are indicated by black arrows.



Supplemental Figure 3A

B

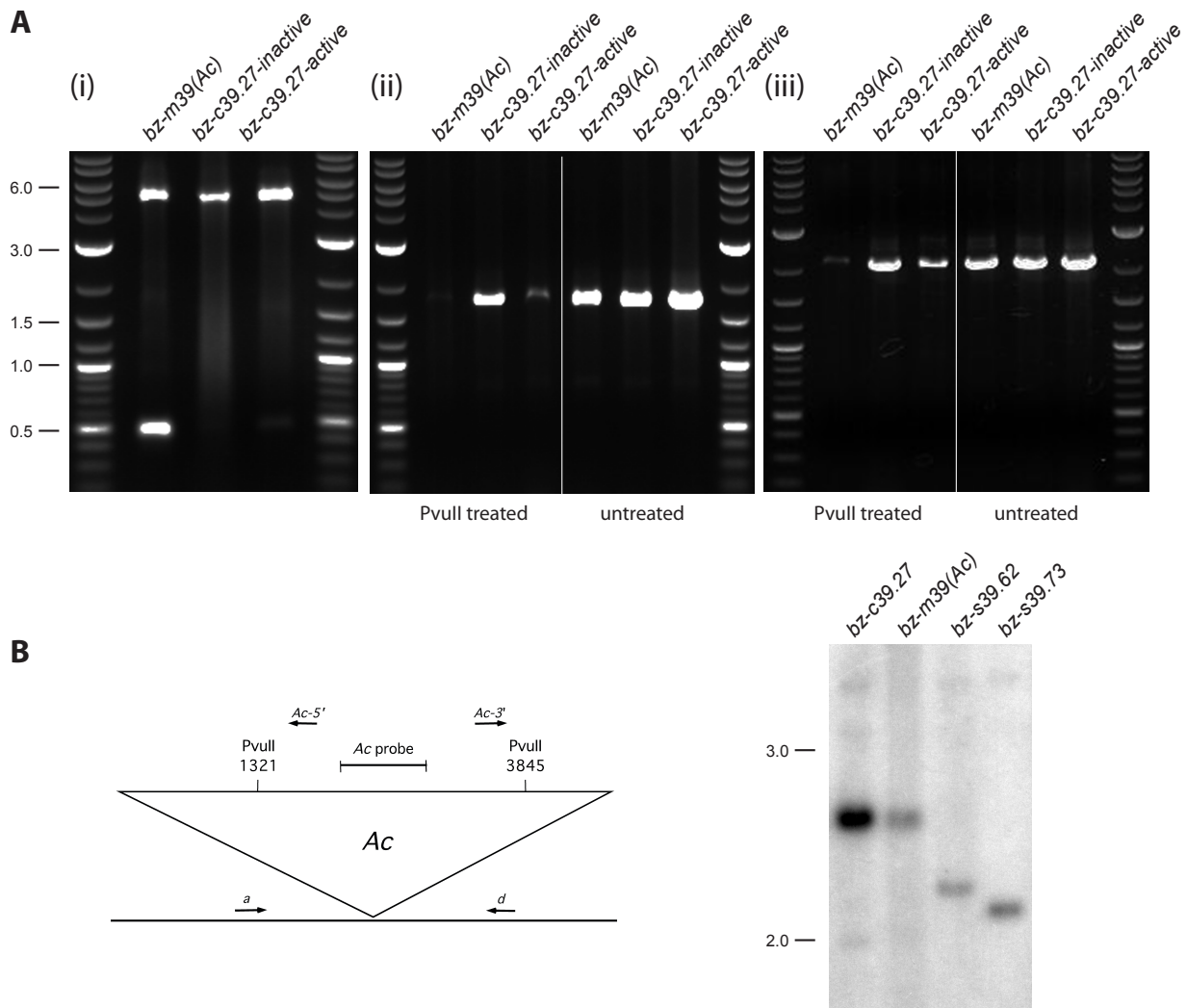
| | |
|------------------------------------|--|
| Ac <i>Z.mays</i> | LEEKE---KLYGKLDVQ--SRFSTTMDMWTs-CQNKSYMCVTHHWIDD----DW-CLQK |
| XP_002444199 <i>S. bicolor</i> | LAEKE---KLYAYLKTVT--CRFSTTMDIWTs-CHNKSYMCVTLHWIDD----NW-HIQK |
| EEE64886 <i>O. sativa</i> | LEEKN---KLYECLKFVK--SRICATMDMWTs-NQNKGYMCVTLHWIDD----NW-RIQK |
| 2021344A_Pac1 <i>P. glaucum</i> | VQEKE---KLYAYFKTVK--SRFSATMDMWTs-NQNKSYMCVTLHWIDD----NW-CIQK |
| AC092172.32 <i>O. sativa</i> | LEEKK---KMYEYFKTLS--CRFCTTMDMWTs-NQNKCYMCITVHWIDD----NW-CMQK |
| AAM97760.1 <i>O. sativa</i> | EVRRK---ILYDELKSVS--SRISTTMDMWTs-NQNKAYMCITAHWIDE----NW-LMQK |
| AEF33496 <i>Saccharum</i> | KNHRT---TLREMFENCN--FRFSLTADLWTs-NQNIIGYMCVTCCHYIDD----DW-KVRK |
| Daysleeper <i>A. thaliana</i> | LAEKQ---NVMKSLGIP--GRVCLTDLFWTs-KLTLGYVFTAHYIDS----DW-KIQK |
| CAN80126 <i>V. vinifera</i> | EFKKG---KMSSYLEKLE--TRMAITTDMWTs-NQKKGYMAITVHYIDE----SW-LLHH |
| XP_002331299 <i>P. trichocarpa</i> | MKEKQ---KVYEMINRLH--GRINLAVEMWSS-PENAEYLCLIAHYIDE----DW-KLQQ |
| Tag2 <i>A. thaliana</i> | EKEKQ---ILKSELERIP--SRICLTSDCWTS-LGGDGYIVLTAHYVDT----RW-ILNS |
| BAA96580.1 <i>O. sativa</i> | ESEKN---QLKSLKEAE--SISLTTDLWTs-NQNLQYMCVTAHFIDE----NW-VMQC |
| XP_003334942 <i>P. graminis</i> | GSMKD---KLIKEIAEV--DRIALTTDLWTs-SNQTFFMVI SAHFISS----DW-TLKN |
| XP_003591965 <i>M. truncatula</i> | DIERL---QLKNFLAEHC--QRVCLTDDMWTsSCQKMSYMCVTAHFIDN----NW-RLHK |
| <i>Mx Z. mays</i> | FOEKA---KLKFFKDFDSC--QRVCLTDDGWTS--QQQDSYMTVTASFIDE----NW-RLHK |
| AC079179.5 <i>O. sativa</i> | EDEKE---VLKFFKDFDNC--VRVCLTDDTWTs-KNSQNFMCVTAHFIDN----NW-NLQK |
| BAJ23914 <i>Saintpaulia</i> | AMEVA---KIKSVIGD---QRISITDDTWTs-IQNIYMVITAHFLDN----DW-KLHK |
| ACX85638.1 <i>C. melo</i> | MKEKK---KLKNALTRSG--QRVCLTDDTWTs-VQNIYMVITAHFIDD----DW-NLHK |
| BAB07989.1 <i>O. sativa</i> | KDRRS---VIIDRLNSA--SSIALTSDIWSG-HAKEDYLSVVAHFVNS----DW-QLEK |
| <i>Tam3 A. majus</i> | EKEII---VLRNEFKNFN--GRISLTSDLWQSG-SGSYHFSCITAHWIDK----DW-IMRK |
| <i>Slide N. tabaccum</i> | HEYEQ---YLRYLFTHIP--NRISITDDIGRS-GNDCDYLTVTSHWIDE----EW-IMQN |
| XP_001223290 <i>C. globosum</i> | EAEKE---LLRAELARSP--YKHLTFDLWTsP-NQYALLGITVHFVDQ----SQ-QLQF |
| EGU87925.1 <i>F. oxysporum</i> | RSQKE---LLRSKLAASL--YKHLTFDLWTsP-QEYALLGVTVHFDTA----LK-RPQT |
| <i>crypt1 C. parasitica</i> | HENLAG---LQLQLQREAI--SKIHL SADLWTsP-NHKGILAVVAHFVDS----DA-KLRN |
| <i>Tascot-1 A. immersus</i> | EREQKH---IVDYFQEHNI--GQINLSFDLWKGPN-NRYYLAVVGHWFDA----KAKKVRH |
| EFY94480 <i>M. anisopliae</i> | EAQKH---QVKRDIQSAL--SKVHFTVDLWTsP-NALAILGIVAHYTSSE----TG-RLKY |
| <i>Tfol F. oxysporum</i> | VGDAGRRVWLMKLVAT--SKIHISVDAWTS E-EGTNYLAVVAHFVDE----SH-KLQT |
| <i>Restless T. inflatum</i> | NGAGK---AVTEHLKTAR--GKIHLAFDGTsR-NQLSLLGVNCFVVDQ----LW-RHRR |
| <i>Tramp H. sapiens</i> | GAVRE---VILKELAEA--TWCGISDTMWRSENQNRAYVTLAAHFLGLGAPNCL-SMGS |
| <i>Hermit L. cuprina</i> | DDKRS---KINEELQMAITSGTASITTDL---NFVKKQFLCVTFHLLIKD----L--KLKE |
| <i>Homer B. tryoni</i> | NEKKE---ELKEEINNIVSSGGASATIDMWTsDNYVKRNFLGVTFFHYQKD----L--KFFD |
| <i>Hobo D. melanogaster</i> | EEKRS---LISSEIKKAVDSGRASATVDMWTDQYVQRNFLGVTFFHYEKE----F--KLCD |
| <i>Hermes M. domestica</i> | KEKKA---LISREIKSAVEKDGASATIDLWTDNYIKRNFLGVTFFHYHEN----N--ELRD |
| <i>I-R Z. mays</i> | -----SIKIVKEAK---YFSVILDCTPD--ISHQEQMTLLVRCINLSNG--KI-NIEE |
| <i>TIP100 I. purpurea</i> | -----AIREETGDAK---FCIIVDEARD--ESKKEQMSIVLRFVDR----DG-FIQE |
| <i>Tag1 A. thaliana</i> | KLQVS---LIIDKFKSSWASTGCTLMADGWKDT-RQRPLINFLVYCPKG----IT--FL |
| <i>Bg Z. mays</i> | YEDME---AHMAKFKDDWKE----- |
| <i>Bg-like O. sativa</i> | ----- |

Supplemental Figure 3B

Supplemental Figure 3. Phylogenetic analysis of *hAT* transposases.

(A) A phylogenetic tree of *hAT* transposases sequences was constructed using MEGA 5.05. Each sequence is identified by either its transposon name or GenBank accession number and the name of its source species. Numbers above the branches indicate the percentage of 1000 bootstrap replications in which that branch was present. The well-supported clades are indicated by brackets and given the name of the first described element(s) in that clade.

(B) Protein sequences were aligned using Clustal Omega. Serine 305 highlighted in yellow is well conserved within the *Ac/Tam3* and *restless* clades.



Supplemental Figure 4

Supplemental Figure 4. Methylation of inactive *Ac* in *bz-c39.27*

A. PCR analysis of *bz-c39.27*.

- (i) PCR assay of *Ac39* excision activity. Primers *a* and *d* were used to amplify the *Ac39* site. The reactivated *bz-c39.27* allele shows a weak 0.5-kb *Ac* empty site band, indicating slight *Ac39* excision activity.
- (ii) Methylation of *Ac39*'s 5' end. Genomic DNA was digested overnight with the methylation sensitive enzyme PvuII and purified using Qiagen QIAEX II kit. Primers *a* and *Ac-D* were used to amplify the *Ac* 5' end fragment containing a single PvuII site. A PCR band similar to the undigested control samples should be produced by samples containing methylated *Ac* elements, but not by samples containing only unmethylated *Ac* elements. The active form of *bz-c39.27* behaves similar to the parental *bz-m39(Ac)*, while the inactive form is hypermethylated and not cut by PvuII.
- (iii) Methylation of *Ac39*'s 3' end. The same DNA templates from (ii) were amplified using primers *Ds-3* and *d* to test the methylation status of the *Ac* 3' end. In agreement with (ii), the inactive *bz-c39.27* is hypermethylated relative to the parental *bz-m39(Ac)* and active *bz-c39.27* alleles.

B. DNA gel blot analysis of *bz* derivatives from *bz-m39(Ac)*.

Methylation sensitive enzyme PvuII digests of genomic DNAs from *bz-m39(Ac)* and *bz* derivatives *bz-c39.27*, *bz-s39.62*, and *bz-s39.74* were blotted into a Hybond XL membrane and hybridized to an *Ac* probe. The source of DNA for each lane is identified above the blot. The locations of the PvuII sites and of the probe in the *Ac* sequence are shown below the map. The *Ac* probe detects a 2.6-kb fragment produced by complete digestion of the PvuII sites in the *bz-m39* progenitor and in the active version of the cycling derivative *bz-c39.27*.

a, 5'-GGGTGTGTCCAGAATGTACCT-3';
b, 5'-GCGTGGCGGCGTGTGAAT-3';
c, 5'-GTGTGCTCCAGATTTATATGGA-3';
d, 5'-AGGACGCGGTGGAGAGGAACGAGAGC-3';
stk1-4, 5'-AACAGGTACACGGCAATGGCAGAG-3';
bz-4R, 5'-CAGTTACCTAGCACTAGAAGAG-3';
LHAcR2, 5'-ATGACAGGCAGCAGCTACG-3';
Ds-A, 5'-CGCCTTCTACTCGCAAAC-3';
Ac-C, 5'-CACACTGGCCAAAGGTTATCACA-3';
Ac-5', 5'-GCTTCTTCTTCGGGTTTCAGGTTGT-3';
Ac-E, 5'-GTGAGGGCGCAGAGACTT-3';
Ds-E, 5'-TCATTGCAACGGCCATTCTCCTAA-3';
Ds-7, 5'-TAGCGCCTCGAGATCACCAA-3';
Ac-3', 5'-ATCTCACTGCATGCGCCTTGTC-3';
Ac-N, 5'-CAACCAAGGCTCATCTGTCAA-3';

Supplemental Table 1. Primers used in this work.