**Supplementary Files**



**Figure S1. Pedigree of *p1-ww::MTn* alleles**

*p1-vv9D9A* gave rise to *Ac::MTn* alleles *P1-rr908, P1-rr458,* and *P1-rr460* by transposition of *Ac* to three sites downstream of the *P1* gene. Subsequent transposition of *Ac::MTn* elements from their positions in *P1-rr458* produced *p1-ww458-4A, p1-ww472-1, p1-ww459-2, p1-ww459-6, p1-ww465-7,* and *p1-ww606-6.* Transposition of *Ac::MTn* element from *P1-rr460* produced *p1-ww460-3B, p1-ww441-7, p1-ww442-6,* and *p1-ww443-3.*



**Figure S2. Methylation status of B73 MTn and its flanking sequences**

The methylation levels of CG, CHG and CHH are shown for MTn and its flanking sequences in B73. The data was extracted from NAM Consortium (Hufford *et al.* 2021).



**Figure S3. Small RNAs mapped to *Ds* elements in B73 MTn.**

The small RNA high-throughput sequencing data analyzed here is under accession number SRP062285 in NCBI (Zuo *et al.* 2016; Wang *et al.* 2020). Small RNA samples are from B73 plant – 14 days after sowing shoot and root. The small RNAs mapped to the self-inserted *Ds* sequences of MTn in B73 are visualized by the Integrative Genomics Viewer. The y axis of the coverage track was standardized to the same scale for each sample. Colors indicate size of sRNA: Black, 21 nt; Red, 22 nt; Blue, 24 nt. The 24 nt small RNAs are enriched in the TIR regions of the *Ds*, which may target MTn and induce or enhance epigenetic silencing of MTn.



**Figure S4. Example sequence changes at excision and insertion sites during *Ac::MTn* transposition.**

The nucleotides flanking *Ac::MTn* are shown in bold in the progenitor alleles *p1-rr458* and *p1-rr460* and red in progeny alleles. Following transposition of *Ac::MTn* , these flanking nucleotides are altered in alleles *p1-ww458-4A* (deletion) and *p1-ww460-3B* (transversions) Target site duplications (TSD) were formed at new *Ac::MTn* insertion site and are underlined in the figure. The position and orientation of newly inserted *Ac::MTn* are also indicated.

458 backbone GCGCTATTGCTCCTACAACTACAA**CG**TTCCCGTCGCGTCGGGACCGGGGCCGGGGCCGAG

458-4A GCGCTATTGCTCCTACAACTACAA--TTCCCGTCGCGTCGGGACCGGGGCCGGGGCCGAG

459-6 GCGCTATTGCTCCTACAACTACAAC-TTCCCGTCGCGTCGGGACCGGGGCCGGGGCCGAG

459-2 GCGCTATTGCTCCTACAACTACAAC-TTCCCGTCGCGTCGGGACCGGGGCCGGGGCCGAG

 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

460 Backbone -------ATATGCGCTATTGCTCCTACAACTACAA**CG**CCGGACATTTATGCTGCCTGCTG

442-6 ---ATAGATATGCGCTATTGCTCCTACAACTACAAGTCCGGACATTTATGCTGCCTGCTG

443-3 ATGATAGATATGCGCTATTGCTCCTACAACTACAAGCCCGGACATTTATGCTGCCTGCTG

460-3B -----AGATATGCGCTATTGCTCCTACAACTACAAGCCCGGACATTTATGCTGCCTGCTG

 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

**Figure S5. Footprint sequences at *Ac::MTn* excision sites.**

The two nucleotides flanking *Ac::MTn* are yellow highlighted in the progenitor alleles *P1-rr458* and *P1-rr460*. These nucleotides are marked in red in *p1-ww::MTn* alleles*.*



**Figure S6. Aberrant Footprint at *Ac::MTn* excision site of *p1-ww465-7*.**

Gel bands from PCR with primers 8 and 9 located on *p1* exon1 (Figure 2A-B). p1 band is absent in *p1-ww465-7.* Positive control: *P1-rr::MTn* and *p1-ww459-6* that carry the intact *p1*; negative control: maize inbred 4Co63 contains *p2* but not *p1*. The absence of p1 in *p1-ww465-7* can be explained by the segregation of the new *Ac::MTn* insertion site from *p1* locus, or a deletion of *p1.*

**

**Figure S7. GC content of *Ac::MTn* flanking sequences in *P1-rrMTn* alleles.**

The percent GC content in 500 bp sequences flanking *Ac::MTn*termini in *P1-rr908, P1-rr458* and *P1-rr460* are shown (numbers under dashed lines). Black boxes indicate *p1* exons 1, 2 and 3 (left to right).

**Table S1. Oligonucleotide primer sequences used in PCR experiments.**

|  |  |
| --- | --- |
| **Primer Number** | **Primer Sequences** |
| 1 | GCTATCAAACAGGACACGGGAGAGAAT |
| 2 | GCTGAATCTGCTTGCCCTGC (for *P1-rr458* and *908*)GCAGCATCCCCGTTACGC (for *P1-rr*460) |
| 3 | TGCCATCTTCCACTCCTCGGCTTTAG |
| 4 | CCCACATGGTGTTGCGAGAG |
| 5 | CCCGTTTCCGTTCCGTTTTCGT |
| 6 | GATTACCGTATTTATCCCGTTCGTTTTC |
| 7 | TTACCTATTGCGGGGGGAGC (for *P1-rr458* and *908*)CGCCCTCGAATCGAAAGCAT (for *P1-rr460*) |
| 8 | ACGCGCGACCAGCTGCTAACCGTG |
| 9 | GAATTCCGCCCGAAGGTAGTTGATCC |
| 10 | GCTCTACCGTTTCCGTTTCCGTTTACCG |
| 11 | CACCACACGAGTAACAGCATCACACATTCAC |
| Bf | GTACCCAAGCGAGGAGGGGAGC |
| Br | CTACTTTGCCCCCCCTCTGGATC |
| P1\_22643f | AGGTCCAACACTCGCTCTTCAT |
| P1\_22927f | CCTCCCTAATGATTTCCCC |
| Ac4508f | ATGAAAATGAAAACGGTAGAGG |

**Table S2. Identification of *Ac::MTn*** **reinsertion events by phenotypic and PCR screenings.**

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\* The number of ears used in phenotypic screening is an estimated number.

“No. of events” means single kernels or multiple kernel sectors with no functional *p1* and with active *Ac*, suggesting an *Ac*-induced loss of function event. The last three columns are from the molecular screening data. *Ac::MTn* excision events are concluded from the footprint PCR. Reinserted/not reinserted events are concluded from the presence or absence of internal structure of *Ac::MTn* in the genome, respectively.

## Supporting Data 1.

## Sequences flanking the reinserted *Ac::MTn* in each allele.

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NNNNNNNGNNNNNCNGNTTTTCGTTTCNTCCCGCAAGTTAAATATGAAAATGAAAACGGTAGAGGTATTTTACCGACCGTTACCGACCGTTTTCATCCCTAGCGGGGAGGTGGAGGTGGAGGTGGAGGCGTTCCGGCAGGGCGCCGTGAAGACGGAGATGTGCAACAAGTGGGAGCGCGGTGTGTGCCCCTACGACGGGCGTTGCAGGTTCGCGCACGGCATGGAGGAGCTGCGCCCCGTGATCCGCCACCCGCGCTACAAGACCCTCCCGTGCCAACTGTTCGCTGCGCCCTCTGGCTGCCCCTACGGCCACCGCTGCCACTTC

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## Supporting Data 2.

## MTn in B73 genome.

TSD flanking MTn

TSD flanking the insertion Ds

Insertion Ds

Host Ds

Flanking sequences

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ATATGTAGCACCTTGAATGACACAAATATGCATCAATATAAGTAAAATAATTGTTGAATAACTATAAATTGGAACTTCATTATAACATATATGCATTCACCTTTTCTAGATGCTGCTACCCAATCTTTTGTGCATATCAAAGCTTCAACAATCTCCGAACCAAGACGATTGCGGTAAGGATCAACAACACGACCACCAGCACTGAACGCAGACTCAGAAGCAACAGTTGACACTTGTATTGCTAGCACATCCCTTGCAATTTGGGTGAGAATAGGATATTCTGCAACCCTTCCCCTCCACCATGATAAAATATCAAACTGACCACTATGCTTCAAAAGGGGTTCAGACATATATTTATCCAATTCATTTGACTCTACTTGATCATAATCCTTCAACTCATGCAAATAGTTTTGAAATTCATCATCTTCATTTTCCATCAAGGTATCATCTATACTATCATTAGTAGTTGTCTTTGTCTTTGGAGCTGAAGGACTACAACTAGAATAGAATTGATACAATTTTCTAATGACCCTAACAAAGTCATCTACATGAACTTTGTATGAATCACCATGGAATTTTTTCATATAGAACTCAATCAATATTTTCTTGTACCTAGGGTCAAGGAAGCATGCTACAGCTAGTGCAATATTAGACACTTTCCAATATTTCTCAAACTTTTCACTCATTGCAACGGCCATTCTCCTAATGACAAATTTTTCATGAACACACCATTGGTCAATCAAATCCTTTATCTCACAGAAACCTTTGTAAAATAAATTTGCAGTGGAATATTGAGTACCAGATAGGAGTTCAGTGAGATCAAAAAACTTCTTCAAACACTTAAAAAGAGTTAATGCCATCTTCCACTCCTCGGCTTTAGGACAAATTGCATCGTACCTACAATAATTGACATTTGATTAATTGAGAATTTATAATGATGACATGTACAACAATTGAGACAAACATACCTGCGAGGATCACTTGTTTTAAGCCTTATTAGTGCAGGCTTATAATATAAGGCATCCCTCAACATCAAATAGGTTGAATTCCGTCTAGTTGAGACATCATATGAGATCCCTTTAGATTTATCCAAGTCACATTCACTAGCACACTTCATTAGTTCTTCCCACTGCAAAGGAGAAGATTTTACAGCAAGAACAATCGCTTTGATTTTCTCAATTGTTCCTGCAATTACAGCCAAGCCATCCTTTGCAACCAAGTTCAGTATGTGACAAGCACACCTCACATGAAAGAAAGCACCATCACAAACTAGATTTGAATCAGTGTCCTGCAAATCCTCAATTATATCGTGCACAGCTACTTCATTTGCACTAGCATTATCCAAAGACAAGGCAAACAATTTTTTCTCAATGTTCCACTTAACCATGATTGCAGTGAAGGTTTGTGATAACCTTTGGCCAGTGTGGCGCCCTTCAACATGAAAAAAGCCAACAATTCTTTTTTGGAGACACCAATCATCATCAATCCAATGGATGGTGACACACATGTATGACTTATTTTGACAAGATGTCCACATATCCATAGTTGTACTGAAGCGAGACTGAACATCTTTAAGTTTTCCATACAACTTTTCTTTTTCTTCCAAATACAAATCCATGTTATATTTTCTAGCAGTGACACGGGACTTTATTGGAAAGTGAGGGCGCAGAGACTTAACAAACTCAACAAAGTACTCATGTTCTACAATATTGAAAGGATATTCATGCATGATTATTGCCAAATGAAGCTTCTTTAGGCTAACCACTTCATCGTACTTATAAGGCTCAATGAGATTTATGTCTTTGCCATGATCCTTTTCACTTTTTAGACACAACTGACCTTTAACTAAACTATGTGATGTTCTCAAGTGATTTCGAAATCCGCTTGTTCCATGATGACCCTCAGCCCTATACTTAGCCTTGCAATTAGGAAAGTTGCAATGTCCCCATACCTGAACGTATTTCTTTCCATCGACCTCCACTTCAATTTCCTTCTTGGTGAAATGCTGCCATACATCCGATGTGCACTTCTTTGCCCTCTTCTGTGGTGCTTCTTCTTCGGGTTCAGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTCATGAACAATAGCCATATCATCTTGACTCGGATCTGTAGCTGTACCATTTGCATTACTACTGCTTACACTCTGAATAAAATGCCTCTCGGCCTCAGCTGTTGATGATGATGGTGATGTGCGGCCACATCCATGCCCACGCGCACGTGCACGTACATTCTGAATCCGACTAGAAGAGGCTTCAGCTTTTCTTTTCAACCCTGTTATAAACAGATTTTTCGTATTATTCTACAGTCAATATGATGCTTCCCAATCTACAACCAATTAGTAATGCTAATGCTATTGCTACTGTTTTTCTAATATATACCTTGAGCATATGAAGAGAATACGGAATTTGTTTTGCGAGTAGAAGGCGCTCTTGTGGTAGACATCAACTTGGCCAATCTTATGGCTGAGCCTGAGGGAGGATTATTTCCAACCGGAGGCGTCATCTGAGGAATGGAGTCGTAGCCGGCTAGCCAAAGTGGAGAGCAGAGCCCCTGGACAGCAGGTGTTCAGCAATCAGCTTGGTGCTGTACTGCTGTGACTTGTGAGCACCTGGACAGCAATCAGCAGGTGTTGCAGAGCCCCTGGACAACACACAACAGCTTGGTGCAATGGTGCTGACGTGCTGTACTGCTAAGTGCTGTGAGCCTGTGAGCAGCCGTGGAGACAGGGAGACCGCGGTCCGCGGATGGCCGGATGGGCGAGCGCCGAGCAGTGGAGGTCTGGAGGACCGCTGACCGCAGATGGCGGATGGCGGATGGCGGATGGGCGGATGGGCGAGCAGTGGAGTGGAGGTCTGGGCGGATGGGCGGACCGCGGCGCGGATGGGCGAGTCGCGAGCAGTGGAGTGGAGGGCGGACCGTGGATGGCGGCGTCTGCGTCCGGCGTGCCGCGTCACGGCCGTCACCGCGTGTGGTGCCTGGTGCAGCCCAGCGGCCGGCCGGCTGGGAGACAGGGAGAGTCGGAGAGAGCAGGCGAGAGCGAGACGCGTCGCCGGCGTCGGCGTGCGGCTGGCGGCGTCCGGACTCCGGCGTGGGCGCGTGGCGGCGTGTGAATGTGTGATGCTGTTACTCGTGTGGTGCCTGGCCGCCTGGGAGAGAGGCAGAGCAGCGTTCGCTAGGTATTTCTTACATGGGCTGGGCCTCAGTGGTTATGGATGGGAGTTGGAGCTGGCCATATTGCAGTCATCCCGAATTAGAAAATACGGTAACGAAACGGGATCATCCCGATTAAAAACGGGATCCCGGTGAAACGGTCGGGAAACTAGCTCTACCGTTTCCGTTTCCGTTTACCGTTTTGTATACCACGTTTCCGTTCCGTTTTCGTTTTTTACCTCGGGTTCGAAATCGATCGGGATAAAACTAACAAAATCGGTTATACGATAACGGTCGGTACGGGATTTTCCCATCCTACTTTCATCCCTAAGTGGAGGTCTGGGCGGATGGGCGGACCGCGGCGCGGATGGGCGAGTCGCGAGCAGTGGAGTGGAGGGCGGACCGTGGATGGCGGCGTCTGCGTCCGGCGTGCCGCGTCACGGCCGTCACCGCGTGTGGTGCCTGGTGCAGCCCAGCGGCCGGCCGGCTGGGAGACAGGGAGAGTCGGAGAGAGCAGGCGAGAGCGAGACGCGTCGCCGGCGTCGGCGTGCGGCTGGCGGCGTCCGGACTCCGGCGTGGGCGCGTGGCGGCGTGTGAATGTGTGATGCTGTTACTCGTGTGGTGCCTGGCCGCCTGGGAGAGAGGCAGAGCAGCGTTCGCTAGGTATTTCTTACATGGGCTGGGCCTCAGTGGTTATGGATGGGAGTTGGAGCTGGCCATATTGCAGTCATCCCGAATTAGAAAATACGGTAACGAAACGGGATCATCCCGATTAAAAACGGGATCCCGGTGAAACGGTCGGGAAACTAGCTCTACCGTTTCCGTTTCCGTTTACCGTTTTGTATACCCCGTTTCCGTTCTGTTTTCGTTTTTTACCTCGGGTTTGAAATCGATCGGGATAAAACTAACAAAATCGGTTATACGATAACGGTCGGTACGGGATTTTCCCATCCTACTTTCATCCCTACCTACGACCAGTAGAATAGCCGAATAATTGGGGTTGCGGCAATGCGTATGGCGAATCAGAACGACCCATGTCAGTAAGCGCTATCGCGATGAAGGAAGCAACCGGAAGCTGTGGTGGACACAGACACAGCCAAGTCCCTCCGTCCAACTTGCCAAGTTGTTCGAG

## Supporting Data 3.

**MTn sequences in the NAM lines**

TSD flanking MTn

TSD flanking the insertion Ds

Insertion Ds

Host Ds

>NC358

CCTACGACTAGGGATGAAAACGGTCGGGAACGGTCGGTAAAATACCTCTACCGTTTTCATTTTCATATTTAACTTGCGGGACGGAAACGAAAACGGGATATACCGGTAACGAAAACGAACGGGATAAATACGGTAATCGAAAACCGATACGATCCGGTCGGGTTAAAGCCGAAATCGGACGGGAACCGGTATTTTTGTTCGGTAAAATCACACATGAAAACATATATTCAAAACTTAAAAACAAATATAAAAAATTGTAAACACAAGTCTTAATTAAACATAGATAAAATCCATATAAATCTGGAGCACACATAGTTTAATGTAGCACATAAGTGATAAGTCTTGGGCTCTTGGCTAACATAAGAAGCCATATAAGTCTACTAGCACACATGACACAATATAAAGTTTAAAACACATATTCATAATCACTTGCTCACATCTGGATCACTTAGCATGCATAAACTATTACAACCAAGGCTCATCTGTCAACAAACGTAAGACACATTGCTCATGGAGAGGAGCCACTTGCTACATCTTCATTATTCTTAGAAAATTCTATTGCGTCTTCATCCTGTTAATACACAAAAATAAGTCAGTTTTGGATAAATAAATACATATAGAAGAACATGAATTGATATGCAGGGAGTATAAATAAATACATATAGGAGAACATGAATCTGTGAACTAACACGGCTGGGAGCTAGGCAGCTAGCAGCTAGCGCCTAACAGCTGGAAGCCTAACAGCTAGCAGCTAGCAGCCAATCAAAACAAGGCGACAAGGCGCATGCAGTGAGATCAAAAATCTGTTAATGCCAGCCATGCAGGGAGTATAACACGGCTGGGCAGCAAGGCGCATGCATCAAAACAAGGCGACAGCAAACAGCCCATGCATCAAAACAGTAGTGAATAATAGCAAATTAATAGCCCATGCACGAAGTAAATAATAATCTTTAAATACCTCATCCATATGATTCTCATGATTTGTTGCAGCAGCAATAACAGAGTCTAGCACCTCGAGATCACCAATCATTGTTGGAAAATATGTAGCACCTTGAATGACACAAATATGCATCAATATAAGTAAAATAATTGTTGAATAACTATAAATTGGAACTTCATTATAACATATATGCATTCACCTTTTCTAGATGCTGCTACCCAATCTTTTGTGCATATCAAAGCTTCAACAATCTCCGAACCAAGACGATTGCGGTAAGGATCAACAACACGACCACCAGCACTGAACGCAGACTCAGAAGCAACAGTTGACACTTGTATTGCTAGCACATCCCTTGCAATTTGGGTGAGAATAGGATATTCTGCAACCCTTCCCCTCCACCATGATAAAATATCAAACTGACCACTATGCTTCAAAAGGGGTTCAGACATATATTTATCCAATTCATTTGACTCTACTTGATCATAATCCTTCAACTCATGCAAATAGTTTTGAAATTCATCATCTTCATTTTCCATCAAGGTATCATCTATACTATCATTAGTAGTTGTCTTTGTCTTTGGAGCTGAAGGACTACAACTAGAATAGAATTGATACAATTTTCTAATGACCCTAACAAAGTCATCTACATGAACTTTGTATGAATCACCATGGAATTTTTTCATATAGAACTCAATCAATATTTTCTTGTACCTAGGGTCAAGGAAGCATGCTACAGCTAGTGCAATATTAGACACTTTCCAATATTTCTCAAACTTTTCACTCATTGCAACGACCATTCTCCTAATGACAAATTTTTCATGAACACACCATTGGTCAATCAAATCCTTTATCTCACAGAAACCTTTGTAAAATAAATTTGCAGTGGAATATTGAGTACCAGATAGGAGTTCAGTGAGATCAAAAAACTTCTTCAAACACTTAAAAAGAGTTAATGCCATCTTCCACTCCTCGGCTTTAGGACAAATTGCATCGTACCTACAATAATTGACATTTGATTAATTGAGAATTTATAATGATGACATGTACAACAATTGAGACAAACATACCTGCGAGGATCACTTGTTTTAAGCCTTATTAGTGCAGGCTTATAATATAAGGCATCCCTCAACATCAAATAGGTTGAATTCCATCTAGTTGAGACATCATATGAGATCCCTTTAGATTTATCCAAGTCACATTCACTAGCACACTTCATTAGTTCTTCCCACTGCAAAGGAGAAGATTTTACAGCAAGAACAATCGCTTTGATTTTCTCAATTGTTCCTGCAATTACAGCCAAGCCATCCTTTGCAACCAAGTTCAGTATGTGACAAGCACACCTCACATGAAAGAAAGCACCATCACAAACTAGATTTGAATCAGTGTCCTGCAAATCCTCAATTATATCGTGCACAGCTACTTCATTTGCACTAGCATTATCCAAAGACAAGGCAAACAATTTTTTCTCAATGTTCCACTTAACCATGATTGCAGTGAAGGTTTGTGATAACCTTTGGCCAGTGTGGCGCCCTTCAACATGAAAAAAGCCAACAATTCTTTTTTGGAGACACCAATCATCATCAATCCAATGGATGGTGACACACATGTATGACTTATTTTGACAAGATGTCCACATATCCATAGTTGTACTGAAGCGAGACTGAACATCTTTAAGTTTTCCATACAACTTTTCTTTTTCTTCCAAATACAAATCCATGATATATTTTCTAGCAGTGACACGGGACTTTATTGGAAAGTGAGGGCGCAGAGACTTAACAAACTCAACAAAGTACTCATGTTCTACAATATTGAAAGGATATTCATGCATGATTATTGCCAAATGAAGCTTCTTTAGGCTAACCACTTCATCGTACTTATAAGGCTCAATGAGATTTATGTCTTTGCCATGATCCTTTTCACTTTTTAGACACAACTGACCTTTAACTAAACTATGTGATGTTCTCAAGTGATTTCGAAATCCGCTTGTTCCATGATGACCCTCAGCCCTATACTTAGCCTTGCAATTAGGAAAGTTGCAATGTCCCCATACCTGAACGTATTTCTTTCCATCGACCTCCACTTCAATTTCCTTCTTGGTGAAATGCTGCCATACATCCGATGTGCACTTCTTTGCCCTCTTCTGTGGTGCTTCTTCTTCGGGTTCAGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTCATGAACAATAGCCATATCATCTTGACTCGGATCTGTAGCTGTACCATTTGCATTACTACTGCTTACACTCTGAATAAAATGCCTCTCGGCCTCAGCTGTTGATGATGATGGTGATGTGCGGCCACATCCATGCCCACGCGCACGTGCACGTACATTCTGAATCCGACTAGAAGAGGCTTCAGCTTTTCTTTTCAACCCTGTTATAAACAGATTTTTCGTATTATTCTACAGTCAATATGATGCTTCCCAATCTACAACCAATTAGTAATGCTAATGCTATTGCTACTGTTTTTCTAATATATACCTTGAGCATATGCAGAGAATACGGAATTTGTTTTGCGAGTAGAAGGCGCTCTTGTGGTAGACATCAACTTGGCCAATCTTATGGCTGAGCCTGAGGGAGGATTATTTCCAACCGGAGGCGTCATCTGAGGAATGGAGTCGTAGCCGGCTAGCCAAAGTGGAGAGCAGAGCCCCTGGACAGCAGGTGTTCAGCAATCAGCTTGGTGCTGTACTGCTGTGACTTGTGAGCACCTGGACAGCAATCAGCAGGTGTTGCAGAGCCCCTGGACAGCACACAACAGCTTGGTGCAATGGTGCTGACGTGCTGTACTGCTAAGTGCTGTGAGCCTGTGAGCAGCCGTGGAGACAGGGAGACCGCGGTCCGCGGATGGCCGGATGGGCGAGCGCCGAGCAGTGGAGGTCTGGAGGACCGCTGACCGCAGATGGCGGATGGCGGATGGGCGGATGGGCGAGCAGTGGAGTGGAGGTAGGGATGAAAACGGTCGGGAACGGTCGGTAAAATACCTCTACCGTTTTCATTTTCATATTTAACTTGCGGGACGGAAACGAAAACGGGATATACCGGTAACGAAAACGAACGGGATAAATACGGTAATCGAAAACCGATACGATCCGGTCGGGTTAAAGCCGAAATCGGACGGGAACCGGTATTTTTGTTCGGTAAAATCACACATGAAAACATATATTCAAAACTTAAAAACAAATATAAAAAATTGTAAACACAAGTCTTAATTAAACATAGATAAAATCCATATAAATCTGGAGCACACATAGTTTAATGTAGCACATAAGTGATAAGTCTTGGGCTCTTGGCTAACATAAGAAGCCATATAAGTCTACTAGCACACATGACACAATATAAAGTTTAAAACACATATTCATAATCACTTGCTCACATCTGGATCACTTAGCATGCATAAACTATTACAACCAAGGCTCATCTGTCAACAAACGTAAGACACATTGCTCATGGAGAGGAGCCACTTGCTACATCTTCATTATTCTTAGAAAATTCTATTGTGTCTTCATCCTGTTAATACACAAAAATAAGTCAGTTTTGGATAAATAAATACATATAGAAGAACATGAATTGATATGCAGGGAGTATAAATAAATACATATAGGAGAACATGAATCTATGAACTAACACGGCTGGGAGCTAGGCAGCTAGCAGCTAGCGCCTAACAGCTGGAAGCCTAACAGCTAGCAGCTAGCAGCCAATCAAAACAAGGCTTCAAGGCGCATGCAGTGAGATCAAAAATCTGTTAATGCCAGCCATGCAGGGAGTATAACACGGCTGGGCAGCAAGGCGCATGCATCAAAACAAGGCGACAGCAAACAGCCCATGCATCAAAACAGTAGTGAATAATAGCAAATTAATAGCCCATGCACGAAGTAAATAATAATCTTTAAATACCTCATCCATATGATTCTCATGATTTGTTGCAGCAGCAATAACAGAGTCTAGCACCTCGAGATCACCAATCATTGTTGGAAAATATGTAGCACCTTGAATGACACAAATATGCATCAATATAAGTAAAA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>B97.ch5

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TAATTGTTGAATAACTATAAATTGGAACTTCATTATAACATATATGCATTCACCTTTTCTAGATGCTGCTACCCAATCTTTTGTGCATATCAAAGCTTCAACAATCTCCGAACCAAGACGATTGCGGTAAGGATCAACAACACGACCACCAGCACTGAACGCAGACTCAGAAGCAACAGTTGACACTTGTATTGCTAGCACATCCCTTGCAATTTGGGTGAGAATAGGATATTCTGCAACCCTTCCCCTCCACCATGATAAAATATCAAACTGACCACTATGCTTCAAAAGGGGTTCAGACATATATTTATCCAATTCATTTGACTCTACTTGATCATAATCCTTCAACTCATGCAAATAGTTTTGAAATTCATCATCTTCATTTTCCATCAAGGTATCATCTATACTATCATTAGTAGTTGTCTTTGTCTTTGGAGCTGAAGGACTACAACTAGAATAGAATTGATACAATTTTCTAATGACCCTAACAAAGTCATCTACATGAACTTTGTATGAATCACCATGGAATTTTTTCATATAGAACTCAATCAATATTTTCTTGTACCTAGGGTCAAGGAAGCATGCTACAGCTAGTGCAATATTAGACACTTTCCAATATTTCTCAAACTTTTCACTCATTGCAACGGCCATTCTCCTAATGACAAATTTTTCATGAACACACCATTGGTCAATCAAATCCTTTATCTCACAGAAACCTTTGTAAAATAAATTTGCAGTGGAATATTGAGTACCAGATAGGAGTTCAGTGAGATCAAAAAACTTCTTCAAACACTTAAAAAGAGTTAATGCCATCTTCCACTCCTCGGCTTTAGGACAAATTGCATCGTACCTACAATAATTGACATTTGATTAATTGAGAATTTATAATGATGACATGTACAACAATTGAGACAAACATACCTGCGAGGATCACTTGTTTTAAGCCTTATTAGTGCAGGCTTATAATATAAGGCATCCCTCAACATCAAATAGGTTGAATTCCATCTAGTTGAGACATCATATGAGATCCCTTTAGATTTATCCAAGTCACATTCACTAGCACACTTCATTAGTTCTTCCCACTGCAAAGGAGAAGATTTTACAGCAAGAACAATCGCTTTGATTTTCTCAATTGTTCCTGCAATTACAGCCAAGCCATCCTTTGCAACCAAGTTCAGTATGTGACAAGCACACCTCACATGAAAGAAAGCACCATCACAAACTAGATTTGAATCAGTGTCCTGCAAATCCTCAATTATATCGTGCACAGCTACTTCATTTGCACTAGCATTATCCAAAGACAAGGCAAACAATTTTTTCTCAATGTTCCACTTAACCATGATTGCAGTGAAGGTTTGTGATAACCTTTGGCCAGTGTGGCGCCCTTCAACATGAAAAAAGCCAACAATTCTTTTTTGGAGACACCAATCATCATCAATCCAATGGATGGTGACACACATGTATGACTTATTTTGACAAGATGTCCACATATCCATAGTTGTACTGAAGCGAGACTGAACATCTTTAAGTTTTCCATACAACTTTTCTTTTTCTTCCAAATACAAATCCATGATATATTTTCTAGCAGTGACACGGGACTTTATTGGAAAGTGAGGGCGCAGAGACTTAACAAACTCAACAAAGTACTCATGTTCTACAATATTGAAAGGATATTCATGCATGATTATTGCCAAATGAAGCTTCTTTAGGCTAACCACTTCATCGTACTTATAAGGCTCAATGAGATTTATGTCTTTGCCATGATCCTTTTCACTTTTTAGACACAACTGACCTTTAACTAAACTATGTGATGTTCTCAAGTGATTTCGAAATCCGCTTGTTCCATGATGACCCTCAGCCCTATACTTAGCCTTGCAATTAGGAAAGTTGCAATGTCCCCATACCTGAACGTATTTCTTTCCATCGACCTCCACTTCAATTTCCTTCTTGGTGAAATGCTGCCATACATCCGATGTGCACTTCTTTGCCCTCTTCTGTGGTGCTTCTTCTTCGGGTTCAGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTGTGGTTCATGAACAATAGCCATATCATCTTGACTCGGATCTGTAGCTGTACCATTTGCATTACTACTGCTTACACTCTGAATAAAATGCCTCTCGACCTCAGCTGTTGATGATGATGGTGATGTGCGGCCACATCCATGCCCACGCGCACGTGCACGTACATTCTGAATCCGACTAGAAGAGGCTTCAGCTTTTCTTTTCAACCCTGTTATAAACAGATTTTTCGTATTATTCTACAGTCAATATGATGCTTCCCAATCTACAACCAATTAGTAATGCTAATGCTATTGCTACTGTTTTTCTAATATATACCTTGAGCATATGAAGAGAATACGGAATTTGTTTTGCGAGTAGAAGGCGCTCTTGTGGTAGACATCAACTTGGCCAATCTTATGGCTGAGCCTGAGGGAGGATTATTTCCAACCGGAGGCGTCATCTGAGGAATGGAGTCGTAGCCGGCTAGCCGAAGTGGAGAGCAGAGCCCCTGGACAGCAGGTGTTCAGCAATCAGCTTGGTGCTGTACTGCTGTGACTTGTGAGCACCTGGACAGCAATCAGCAGGTGTTGCAGAGCCCCTGGACAACACACAACAGCTTGGTGCAATGGTGCTGACGTGCTGTACTGCTAAGTGCTGTGAGCCTGTGAGCAGCCGTGGAGACAGGGAGACCGCGGTCCGCGGATGGCCGGATGGGCGAGCGCCGAGCAGTGGAGGTCTGGAGGACCGCTGACCGCAGATGGCGGATGGCGGATGGGCGGATGGGCGAGCAGTGGAGTGGAGGTCTGGGCGGATGGGCGGACCGCGGCGCGGATGGGCGAGTCGCGAGCAGTGGAGTGGAGGGCGGACCGTGGATGGCGGCGTCTGCGTCCGGCGTGCCGCGTCACGGCCGTCACCGCGTGTGGTGCCTGGTGCAGCCCAGCGGCCGGCCGGCTGGGAGACAGGGAGAGTCGGAGAGAGCAGGCGAGAGCGAGACGCGTCGCCGGCGTCGGCGTGCGGCTGGCGGCGTCCGGACTCCGGCGTGGGCGCGTGGCGGCGTGTGAATGTGTGATGCTGTTACTCGTGTGGTGCCTGGCCGCCTGGGAGAGAGGCAGAGCAGCGTTCGCTAGGTATTTCTTACATGGGCTGGGCCTCAGTGGTTATGGATGGGAGTTGGAGCTGGCCATATTGCAGTCATCCCGAATTAGAAAATACGGTAACGAAACGGGATCATCCCGATTAAAAACGGGATCCCGGTGAAACGGTCGGGAAACTAGCTCTACCGTTTCCGTTTCCGTTTACCGTTTTGTATACCCCGTTTCCGTTCCGTTTTCGTTTTTTACCTCGGGTTCGAAATCGATCGGGATAAAACTAACAAAATCGGTTATACGATAACGGTCGGTACGGGATTTTCCCATCCTACTTTCATCCCTAAGTGGAGGTCTGGGCGGATGGGCGAGTCGCGAGCAGTGGAGTGGAGGGCGGACCGTGGATGGCGGCGTCTGCGTCCGGCGTGCCGCGTCACGGCCGTCACCGCGTGTGGTGCCTGGTGCAGCCCAGCGGCCGGCCGGCTGGGAGACAGGGAGAGTCGGAGAGAGCAGGCGAGAGCGAGACGCGTCGCCGGCGTCGGCGTGCGGCTGGCGGCGTCCGGACTCCGGCGTGGGCGCGTGGCGGCGTGTGAATGTGTGATGCTGTTACTCGTGTGGTGCCTGGCCGCCTGGGAGAGAGGCAGAGCAGCGTTCGCTAGGTATTTCTTACATGGGCTGGGCCTCAGTGGTTATGGATGGGAGTTGGAGCTGGCCATATTGCAGTCATCCCGAATTAGAAAATACGGTAACGAAACGGGATCATCCCGATTAAAAACGGGATCCCGGTGAAACGGTCGGGAAACTAGCTCTACCGTTTCCGTTTCCGTTTACCGTTTTGTATACCCCGTTTCCGTTCTGTTTTCGTTTTTTACCTCGGGTTTGAAATCGATCGGGATAAAACTAACAAAATCGGTTATACGATAACGGTCGGTACGGGATTTTCCCATCCTACTTTCATCCCTACCTACGAC

## Supporting Data 4.

## Alignment of MTn element in B73 and 5 NAM linesTable  Description automatically generated with medium confidence

## Supporting Data 5.

## Statistical Comparison of Target Site Duplications generated by *Ac::MTn* and *Ds*

Statistical analysis was performed to compare the frequencies of perfect and imperfect TSDs generated by *Ac::MTn* and *Ds* insertions. The *Ds* TSD data are derived from *Ds* transpositions from *a1* locus in maize.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ***Ac::MTn*** | ***Ds*\*** | Row Total |
| **Perfect TSD** | **6** | **97** | 103 |
| **Imperfect TSD** | **2** | **12** | 14 |
| Total | 8 | 109 | 117 |

The 2 x 2 Contingency table containing the *Ac::MTn* and *Ds* TSD data. Perfect TSDs are defined as identical 8 bp duplicated sequences flanking a newly inserted TE (*Ac::MTn* or *Ds*). An Imperfect TSD is defined as the absence of a Perfect TSD; i.e., the presence of one or more missing or non-identical bp in the 8 bp sequence flanking a newly inserted TE.

\*(Vollbrecht *et al.* 2010) and personal communication with Dr. Erik Vollbrecht and Dr. Justin Schares.

The null hypothesis (Ho) is that *Ac::MTn* and *Ds* are equally likely to have perfect TSDs. The results obtained are p = 0.2446 by Fisher-Exact Test, and p = 0.172078 from the Barnard’s Test. Both tests show p > 0.05, indicating that Ho cannot be excluded. Based on these data, we find no evidence that the frequency of perfect and imperfect TSDs differs between *Ac::MTn* and *Ds* transpositions.

## Supporting Data 6.

## The *Ac::MTn* termini are intact in *P1-rr908.*

*Ac* termini sequences

Target site duplication

>908fAc\_flanking

NNNTCCNTCGTGCATGCATGCCACTGTAGCGCCGTAATATAATGATAGATATGCGCTATTGCTCCTACAACTACAACTAGGGATGAAAACGGTCGGTAACGGTCGGTAAAATACCTCTACCGTTTTCATTTTCATATTTAACTTGCGGGACGGAAACGAAAACGGGATATACCGGTAACGAAAACGAACGGGATAAATACGGTAATCA

>908Ac\_3

CNNNNNNNNNNNNNNNNGAGNNACACGGGNTGAGGGTTTTGGTGAGCCCGTGAAGCGCGCCGTAGCCGCGGGAGNTGAAACCCAGGCAACCCAGGAGGCCCTGCCGGTAGGTAGGGATGAAAACGGTCGGTAACGGTCGGTAAAATACCTCTACCGTTTTCATTTTCATATTTAACTTGCGGGACGGAAACGAAAACGGGATATACCGGTAACGAAAACGAACGGGATAAATACGGTAATCGAAAACNGATACGATCCGGNCGGGTTAAAGTCGAAATCGGACGGNAACCGGTATTTTTGTTCGGTAAAATCACNCATGAAAACATATATTCAAAACTTAAAAACAAATATAAANACTTGGNATCGGAGTCTAANNTATGCATAGATAAAATCCAATATGCCNGGNNCCACNAGTTTAATTCAGCACATANGTAACA

>908Ac\_5

GAAACGGGATCATNCCCGATTAAAAACNGNATNNNNNNNGAANANNTCGGNNANCTAGCTNTACCGNNNNNGTNTACGTTGACCGTTTTGTATATCCCGTTTCCGNTCCGTTTTCGTTNTTTACCTCGGGTTCGAAATCGATCGGGATAAAACTAACAAAATCGGTTATNCGATAACGGTCGGTACGGGATTTTCCCATCCTACTTTCATCCCTGCCGGTAGGAAGGAACGTGCGCCGCCGCATCTCCTCCGCCTGCGCACGGTTCGGCGCCGCGTAGAGCCTCTCGTGCGCCGCCGCCACCGTAGTGGAACGTCGCCGCCTGCCCCGGCCCCGGCCCCTACCCTGCCCCCGCGGCCGGCCATCCTCG

## Supporting Data 7.

## GC content of sequences flanking *Ac::MTn* termini*.*

>fAc\_flanking **(GC%=37%)**

Atagtatttataatacccaaaaaaacaatgtcatggcatttattaacaacgggttttcattaaattaaactgatttggtttgccgtaaatttaggagaaccaatatgatcaatgagtagcgtatatcttacagtacagaaaaatctttgaaaaaaatatataaacagcacacaaaagcatgcgattcgtcgacagcatcgtctcacggtctcaccacaccattcaaggacgatggagcttcttcggtgctcagactttatttctaccatctacaacccaaactgatatgtacagtaaatggaaggaagaacaagatagagaaaaaaaacccaaactgatagattagaagtcgctgaagaaatcatctaacaaaactggcgagctatcaaacaggacacgggagagaatagatgattaaacaataatccctcgtgcaatgcatgccactgtagcgccgtaatataatgatagatatgcgctattgctcctacaactacaac

>458Ac\_flanking **(GC%=75%)**

Gtggaggaggacgaggacgagcgggacgaggcggttaccggcggggctgcctccccgtgcgttcccgtcgcgtcgggaccggggccggggccgagatccggcgccgggacgacgagcgcattgatcagcttgagccgcggtgacgcgctgcggagcggggacatggaccgcgcgcggcggtgcatggacgcgctccggggcgtgaggtcgcggccgcgcgtcggcgcgcggccgcccacgcagtcctcgtcgtcgtcgcttccggggtcgagatccgggagcgggggcagggtgagtggccggatctggccgttgtggaagagctcgtcggcggagatcatgggcccgccaggcccgccgaactcgaactccgcgtcgcccccgcacccacgaacaccggagctgggggtcgcggaggcggagcacgaggtcgagaggaggaggtggtggatggggctggccggcgcgctgaagaagtagccgccgccgccgccgccgag

>460Ac\_flanking **(GC%=58%)**

Gccggacatttatgctgcctgctgggagagtcgatccgagagtggtacattaaaaacgcacgctagaattggtgctttcgaacttggggagccggggggccccgttctgccgttcgatccaagcggcacgggaggctgaggcgcgggccaggagctggactgggccccacctggctgtggtttggaaacgggcaacggctctttttaaacacggcattttcaaattctggtcgacgcggcatgcgactcacgagagggcacacgccgcgcggatcttgatgcgtgcgatggacggttgtgacgggtggtatagcggagtggttgggttgtctccgtacgagaaaagctgtcgctctactagtctactacgatttggaacagtaaacagtgtggccactggcgtggcctcacgcgattgggtgacccggctagcgattcgacagagctctgtgtggactgcggaggcgagatggtgagaactgagcccgactagtttgaaa

>908Ac\_flanking **(GC%=75%)**

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