# The maize W22 genome provides a foundation for functional genomics and transposon biology 

Nathan M. Springer¹, Sarah N. Anderson $\odot^{\bullet}{ }^{1}$, Carson M. Andorf ${ }^{2}$, Kevin R. Ahern $\odot^{3}$, Fang Bai $\odot^{4}$, Omer Barad ${ }^{5}$, W. Brad Barbazuk ${ }^{6}$, Hank W. Bass $\oplus{ }^{7}$, Kobi Baruch ${ }^{5}$, Gil Ben-Zvi $\odot^{5}$, Edward S. Buckler $\odot^{\text {8,9, }}$, Robert Bukowski', Michael S. Campbell ${ }^{10}$, Ethalinda K. S. Cannon $~^{2}{ }^{2}$, Paul Chomet ${ }^{5}$, R. Kelly Dawe ${ }^{11}$, Ruth Davenport ${ }^{6}$, Hugo K. Dooner ${ }^{12,13}$, Limei He Du ${ }^{12,13}$, Chunguang Du ${ }^{14}$, Katherine A. Easterling ${ }^{7}$, Christine Gault ${ }^{6}$, Jiahn-Chou Guan ${ }^{4}$, Charles T. Hunter ${ }^{15}$, Georg Jander $\oplus^{3}$, Yinping Jiao ${ }^{10}$, Karen E. Koch ${ }^{4}$, Guy Kol', Tobias G. Köllner $\odot^{16}$, Toru Kudo ${ }^{4,17}$, Qing Li', Fei Lu $\odot^{9,18,19}$, Dustin Mayfield-Jones ${ }^{20}$, Wenbin Mei $\odot^{6}$, Donald R. McCarty ${ }^{4}$, Jaclyn M. Noshay', John L. Portwood II², Gil Ronen ${ }^{5}$, A. Mark Settles ${ }^{( }{ }^{4}$, Doron Shem-Tov ${ }^{5}$, Jinghua Shi ${ }^{21}$, Ilya Soifer ${ }^{5}$, Joshua C. Stein ${ }^{10}$, Michelle C. Stitzer ${ }^{22}$, Masaharu Suzuki ${ }^{4}$, Daniel L. Vera $\oplus^{23}$, Erik Vollbrecht ${ }^{24}$, Julia T. Vrebalov ${ }^{3}$, Doreen Ware $\odot^{8,10,25}$, Sharon Wei ${ }^{10}$, Kokulapalan Wimalanathan $\oplus^{24}$, Margaret R. Woodhouse ${ }^{2}$, Wenwei Xiong $\oplus^{14}$ and Thomas P. Brutnell $\odot^{20,26 *}$

[^0]A
BUSCO Analysis of W22 and B73 genomes


| B |  |  |
| :--- | :--- | :--- |
| Gene Type | W22v2 | B73v4 |
| Complete Single-Copy BUSCOs | 1307 | 1302 |
| Complete Duplicated BUSCOs | 75 | 81 |
| Fragmented BUSCOs | 16 | 18 |
| Missing BUSCOs | 42 | 39 |
| Total BUSCO groups searched | 1440 | 1440 |

## Supplementary Figure 1

## Comparison of the completeness of the B73v4 and W22v2 genome annotations.

The completeness of the genome assemblies and genome annotations was assessed by benchmarking a universal single-copy orthologous gene set (BUSCO) (Simão et al. 2015). The relative frequencies of complete single-copy genes, duplicated genes, fragmented genes and missing genes were very similar in the B73v4 and W22v2 genomes.

## Shared and Unique Alternative Splicing of W22 Compare to B73v4



## Supplementary Figure 2

## Density plot of shared and unique alternative splicing of W22 compared to B73v4.

For the B73v4 alternative splicing events, we mapped the isoforms from B73v4 annotation to the W22 genome and identified the alternative splicing events. We classified the common alternative splicing events based on the coordinates of the alternative region relative to the W22v2 genome.


## Supplementary Figure 3

## Improved transcriptome analyses using the W22v2 genome.

a, RNA-seq data derived from endosperm tissue of W22 (SRA: SRR1986376) were aligned to both B73v4 and W22v2 using the same parameters. The percentage of mapped reads improves by mapping the data to W22. b, A set of 20,994 syntenic orthologous genes in B73 and W22 were identified and used for comparison of expression levels in alignments to B73 or W22. A comparison of expression level (reads per million, RPM) shows generally similar estimates in both genotypes with some genes that have differing expression estimates depending upon which genome was used for a reference. c-f, Differences in RPKM estimates can result from differences in annotation or mapping efficiency. c,d, Overlapping gene models in B73 result in all reads mapping to Zm 00001 d 028756 (orange transcript) to be called ambiguous, while the corresponding gene in W22, Zm00004b001227 (orange transcript), has reads assigned. The adjacent gene, Zm00004b001228 in W22 and Zm00001d028757 in B73 (teal transcripts), has the same number of reads assigned to each reference, with a lower RPKM value reported in B73 owing to the longer gene model. e,f, The RPKM value for genes

Zm00004b005439 in W22 and Zm00001d034453 in B73 is higher when mapping to W22 (e) than to B73 (f) owing to improved alignment to several regions of the gene (marked in yellow). Mapped reads are colored by strand: blue, forward; red, reverse.


## Supplementary Figure 4

## Comparison of TIR copy number in B73 and W22.

a, The proportion of TIR families in each of the superfamilies (Activator (DTA); CACTA (DTC), PIF/Harbinger (DTH), Mutator (DTM), Tourist (DTT)) was determined for all TIR families (blue) in the B73 and W22 genomes. The proportion of TIR families in these categories was then determined for B73-specific (orange) and W22-specific (gray) families. b, The copy number in each TIR TE family is shown for B73 and W22. Color indicates superfamilies. c, The relative copy number for each LTR TE family in B73 and W22 is shown. In d, only families with $<500$ copies are shown. e, Boxplot of the percent identity of LTR sequences for LTR retrotransposons, demonstrating that elements in B 73 -specific families with at least five members (orange) are younger than members of shared families (blue) and elements in B73-specific families with fewer than five members (red). Line, median; box limits, first and third quartiles; whiskers, furthest point within 1.5 * IQR; points, outliers.


## Supplementary Figure 5

Profiles of CHH methylation surrounding sites targeted by Ds (solid lines) or Mu (dashed lines).
The level of CHH methylation is shown for the flanking regions (up to 10 kb ) near $D s$ or $M u$ insertion sites.


## *likely non-functional proteins

## Supplementary Figure 6

## Dendrogram analysis (unrooted tree) of terpene synthases in B73 and W22.

A set of 81 amino acid sequences ( 42 from B73 in blue and 39 from W22 in black) were used to generate a tree based on MUSCLE protein alignment by using the Maximum Likelihood method and a previously described substitution model. Bootstrap values ( $n=1,000$ replicates) are shown next to each node. The tree is drawn to scale, with branch lengths measured in the number of substitutions per
site. All positions with less than $80 \%$ site coverage were eliminated. Evolutionary analyses were conducted in MEGA6. Asterisks are used to mark likely non-functional genes.


## Supplementary Figure 7

## Sequence conservation among Mutator transposable elements.

a, Diagram of a Mutator transposable element. The highly conserved terminal inverted repeats (TIRs) can be used to identify Mu elements and to categorize them based on phylogenetic groups. b, Alignment of consensus sequences for each of the seven Mu TIR groups. Highlighted nucleotides indicate disagreements with the overall consensus sequence. The terminal 20 positions are the most conserved across TIR groups. The predicted transposase-binding site (from position 34 to 68 ) is also highly conserved (57\%) across the seven groups.


## Supplementary Figure 8

Analysis of synteny between Mutator transposons identified in W22 and B73, including intact elements and orphan TIRs. Of the 386 Mu elements or orphan TIRs examined, 133 were present in both the W22 and B73 genomes.

## Supplementary Note

## Characterization and screening of gene models:

The "working set" of gene models ( $\mathrm{n}=40,690$ loci) was subjected to several analyses to distinguish high-confidence genes from transposon-encoded loci and other dubious annotations. MAKER-P calculates an annotation evidence distance (AED) for each model that scores how well the model is supported by its evidence (a range between 0 and 1 , with lower scores indicating higher support) ${ }^{1}$. To gain greater knowledge of putative function of annotated loci, all predicted proteins were annotated using InterProScan (v5) ${ }^{2}$, following default parameters.

Screens for transposon-encoded genes: Probable transposable element (TE) genes were identified using two screens. First, we tagged loci whose longest predicted coding region (CDS) overlapped with repeat-masked regions by more than $40 \%$ of length. Such annotations can arise from from evidence that seeded in non-masked regions but subsequently extended into masked regions. Second, loci with the following InterPro domains were tagged as probable TE: IPR000477, IPR004252, IPR004264, IPR004332, IPR005162, IPR007321, IPR008906, IPR009227, IPR013103, IPR013242, IPR018289, IPR025476, IPR026960, IPR027806.

Comparative genomics analysis: Sequence homology and conserved synteny within related species is suggestive of genetic function and can provide a measure of confidence in the validity of predicted genes. We applied the Ensembl Compara phylogenetic gene tree pipeline ${ }^{3,4}$ to define homologies within the W22 working set and identify orthologous and paralogous relationships with related grass and other plant species. Additional representative genome annotations included those of Zea mays (B73 RefGen_v4), Oryza sativa (IRGSP-1.0), Sorghum bicolor (JGI v2.0), Setaria italica (JGI v2.0), Brachypodium distachyon (JGI v1.0), and three dicot species, Arabidopsis thaliana (TAIR10), Glycine max (JGI v1.0), and Vitis vinifera (CRIBI

V1). The analysis was performed with Ensembl software release 86; online documentation provides further details of the protocol used ("Protein Trees and Orthologies" 2017). Synteny maps relating collinear or near-collinear orthologous genes were constructed between all pairwise combinations of W22, B73, rice, sorghum, Setaria, and Brachypodium using previously described methods ${ }^{5,6}$. This enabled the categorization of W22 annotations, after excluding probable TE, into the following classes based on evolutionary conservation, 1) syntelogs (having conserved ancestral chromosomal position with orthologs in another grass species), 2) synteny with B73 only (which may include loci from maize-specific families), 3) non-syntenic orthologs (having orthologs at non-conserved position in other grasses), and 4) non-orthologs (including W22-specific and maize-specific loci).

Fragmented loci: Putative fragmented loci, which may represent pseudogenes or artifacts from incorrect annotation or misassembly, were identified in two screens. First, we identified gene models that appeared to lack a complete CDS, by absence of a methionine start codon or a stop codon, in all of its transcript isoforms. Second, for those models having an ortholog in B73 or other grass, we looked for extreme deviations of its predicted longest protein length from the average coding length of its orthologs. Those with a z-score less than -2 (e.g. length greater than two standard deviations shorter than the ortholog mean) were also tagged as putative fragmented loci.

## Analysis of local duplications in W22 and B73

The frequency of locally duplicated genes is comparable in B73 and W22, but W22 (14.73\%) had slightly more than B73 (~14.08\%) (Supplementary Table 3). Both genomes had more duplicated genes in tandem (i.e., no intervening genes) ( $\sim 63.9 \%$ in B73 and $\sim 56.4 \%$ in W22) compared to the sum of all other local duplication classes (i.e., with 1 to 20 intervening genes) (Supplementary Table 3). B73 had more tandemly duplicated genes, but W22 had more genes
in other locally duplicated classes. Moreover, the proportional increase in the number of nontandem locally duplicated genes in W22 genome compared to B73 is positively correlated to the number of intervening genes between local duplication events (Supplementary Table 3). In many cases locally duplicated genes form arrays of similar genes, which indicates that a single ancestral gene has been copied multiple times. The current analysis cannot determine the nature and timing of these multiplication events, but it can delineate the current number of gene copies in each multiplication cluster. The overall number of multiplication clusters is comparable between W22 and B73 (~27\% of total multiplication events), although W22 had slightly more (2.3\%) clusters (Supplementary Table 3). As the number of gene copies in a cluster increases, the number of clusters decreases (Supplementary Table 4). Only a few clusters have more than 10 copies, and the highest cluster sizes are 21 for W22 and 20 for B73. Tandem duplicated genes were also classified based on the master list of ortholog mappings between W22 and B73, which revealed that B73 had a higher number (133 duplications or $\sim 6.86 \%$ more) of tandem duplications (Supplementary Table 5), and that fewer tandem duplications are shared between genomes than are unique to one or the other (Supplementary Table 5). Unique tandem duplications predominate and could reflect PAVs or genes that have diverged beyond recognition by the current methodology. A larger proportion of both the shared and unique tandem duplications were in the same (head-tail) orientation, which means that both genes are in the same strand, whereas the number of divergent (head-head) and divergent (tail-tail) are comparable, and both these orientations mean that the genes are not on the same strand.

## Example of functional implications of local duplication for terpene synthase

The terpene synthases of B73 and W22 were assessed in detail (Supplementary Figure 6). The analysis involved 81 amino acid sequences, 42 predicted terpene synthases from B73 and 39 from W22. Protein sequences consisting of less than 500 amino acids, which are likely to be non-functional ${ }^{7}$, are included in the analysis and are marked with asterisks in Figure S6. B73
terpene synthases are shown in blue and W22 terpene synthases are shown in black.

There is a one-to-one correspondence of B73 and W22 copalyl diphosphate synthases and kaurene synthases, with no obviously non-functional proteins. However, mono- and sesquiterpene synthases show significant variation between the two inbred lines. Based on having less than 500 amino acids in the predicted protein length, 13 of the 30 B73 proteins and 12 of the 27 W22 proteins may be non-functional. Although some of these shortened proteins may be the result of incorrect annotation, non-functional terpene synthase pseudogenes have been identified previously in maize. Six terpene synthases are present in B73 but are absent or probably non-functional in W22. Three terpene synthases are present in W22 but probably nonfunctional in B73. Six terpene synthases are likely to be non-functional in both B73 and W22. This relatively large amount of genetic variation between two maize inbred lines is likely reflective of a much greater diversity in the biosynthesis of mono- and sesquiterpenes in maize as a species.

The TPS2/TPS3 sub-tree provides an example where genetic variation facilitated the identification of a knockout mutation for investigating in vivo protein function. B73 has tandemduplicated TPS2 and TPS3 genes, which encode two proteins with $95 \%$ identity at the amino acid sequence level that catalyze the synthesis of linalool, (E)-nerolidol, and (E,E)geranyllinalool ${ }^{8}$. In contrast, W22 has only one such gene, Zm 00004 b 012724 , which is similar to TPS3. The more TPS2-like Zm00004b012719 is a truncated pseudogene in W22. Due to this natural gene knockout in W22, it was possible to identify a Ds transposon knockout mutation of Zm00004a053478, thereby confirming not only the in vivo function in terpene production, but also a role for this enzyme activity in maize-insect interactions ${ }^{9}$.

Mutator ( Mu ) transposable elements are best classified by their highly conserved terminal inverted repeats (TIRs) due to extensive divergence among internal sequences ${ }^{10-12}$ (Supplementary Figure 7). Here, we used known TIRs of Mu elements to query the B73 (v4) and W22 genomes. Phylogenetic analyses revealed 7 distinct clades of Mu TIRs, termed Group 1 through Group 7 (Supplementary Table 7). The Group-1 TIRs (96 in B73 and 99 in W22) included those from the mobile $M u$ elements in $M u$-active populations derived from Robertson's Mutator ${ }^{13-16}$. Also in Group 1 are all but one of the $M u$ elements previously designated "Mu1 through Mu18" ${ }^{17-25}$. The exception was "Mu12" ${ }^{26}$, which has TIRs of phylogenetic Group 2. Consensus sequences for each group were generated by MUSCLE alignment ${ }^{27}$ and are diagrammed in Supplementary Figure 7B. The predicted transposase binding site ${ }^{28}$ is conserved ( $57 \%$ of nucleotides are identical across all seven TIR groups between positions 34 and 68). When these clade-specific TIR consensus sequences were used to query the B73 (v4) and W22 (v2) genomes, the two inbreds were found to have similar numbers of $M u$-element TIRs across phylogenetic groups, as well as total Mu TIRs (Supplementary Table 7).

Individual TIRs within each genome were manually assigned partners (left and right arms) based on proximity to one another and on the presence of matching target-site duplications (TSDs) produced during Mu-element insertion (Supplementary Table 8). The majority (89\%) of TIRs could be paired, resulting in intact $M u$ elements with left and right arms. The remaining "orphan" TIRs represent either TIRs that have lost a recognizable partner, or TIRs that occur as tandem duplications within an intact element. Synteny of $M u$-element insertion sites between W22 and B73 was examined by comparing TSD sequences, TIR group ID's, and chromosome assignments for each Mu element and orphan TIR. Of the 257 Mu elements in W22, approximately half (133) were syntenic with B73 (Supplementary Figure 8).

Although the abundance and types of Mutator $(M u)$ transposable elements in B73 and W22 are similar, the individual identities and locations of $M u$ insertions in these genomes differ substantially. Both B73 and W22 carry comparable numbers of $M u$ transposons and also similar proportions of $M u$ insertions belonging to the seven, phylogenetically-distinct clades or "groups" (Supplementary Table 7, Supplementary Figure 8). Together, this conservation of total $M u$ numbers and their consistent phylogenetic distribution (Supplementary Table 6, Supplementary Figure 8) indicate that the observed pattern predated development of separate inbreds. However, differences in identity and location of individual $M u$ elements (Supplementary Table 8) are consistent with the probable diversity of $M u$ transposons present in the common ancestor of the two inbreds. This inference is consistent with a shared synteny of approximately $50 \%$ for specific Mu-insertions in both B73 and W22 (Supplementary Figure 8). It is tempting to speculate that the extent of non-syntenic Mu sites may correlate with other measures of genome diversity.

## Supplementary Note references

1. Law, M. et al. Automated update, revision, and quality control of the maize genome annotations using MAKER-P improves the B73 RefGen_v3 gene models and identifies new genes. Plant Physiol. 167, 25-39 (2015).
2. Finn, R. D. et al. InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res. 45, D190-D199 (2017).
3. Herrero, J. et al. Ensembl comparative genomics resources. Database 2016, (2016).
4. Vilella, A. J. et al. EnsembICompara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. Genome Res. 19, 327-335 (2009).
5. Schnable, P. S. et al. The B73 maize genome: complexity, diversity, and dynamics. Science 326, 1112-1115 (2009).
6. Youens-Clark, K. et al. Gramene database in 2010: updates and extensions. Nucleic Acids

Res. 39, D1085-94 (2011).
7. Degenhardt, J., Köllner, T. G. \& Gershenzon, J. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry 70, 1621-1637 (2009).
8. Richter, A. et al. Characterization of Biosynthetic Pathways for the Production of the Volatile Homoterpenes DMNT and TMTT in Zea mays. Plant Cell 28, 2651-2665 (2016).
9. Tzin, V. et al. Dynamic Maize Responses to Aphid Feeding Are Revealed by a Time Series of Transcriptomic and Metabolomic Assays. Plant Physiol. 169, 1727-1743 (2015).
10. Chandler, V. L. \& Hardeman, K. J. The Mu elements of Zea mays. Adv. Genet. 30, 77-122 (1992).
11. Bennetzen, J. L. The Mutator transposable element system of maize. Curr. Top. Microbiol. Immunol. 204, 195-229 (1996).
12. Lisch, D. Mutator transposons. Trends Plant Sci. 7, 498-504 (2002).
13. Walbot, V. Saturation mutagenesis using maize transposons. Curr. Opin. Plant Biol. 3, 103-107 (2000).
14. May, B. P. et al. Maize-targeted mutagenesis: A knockout resource for maize. Proc. Natl. Acad. Sci. U. S. A. 100, 11541-11546 (2003).
15. Settles, A. M. et al. Sequence-indexed mutations in maize using the UniformMu transposon-tagging population. BMC Genomics 8, 116 (2007).
16. Williams-Carrier, R. et al. Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. Plant J. 63, 167-177 (2010).
17. Robertson, D. S. Characterization of a mutator system in maize. Mutat. Res./Fundam. Mol. Mech. Mutag. 51, 21-28 (1978/7).
18. Bennetzen, J. L. Transposable element Mu1 is found in multiple copies only in Robertson's Mutator maize lines. J. Mol. Appl. Genet. 2, 519-524 (1984).
19. Taylor, L. P. \& Walbot, V. Isolation and characterization of a 1.7-kb transposable element
from a mutator line of maize. Genetics 117, 297-307 (1987).
20. Oishi, K. K. \& Freeling, M. A New Mu Element from a Robertson's Mutator Line. in Plant Transposable Elements 289-291 (Springer, Boston, MA, 1988).
21. Talbert, L. E., Patterson, G. I. \& Chandler, V. L. Mu transposable elements are structurally diverse and distributed throughout the genusZea. J. Mol. Evol. 29, 28-39 (1989).
22. Fleenor, D., Spell, M., Robertson, D. \& Wessler, S. Nucleotide sequence of the maize Mutator element, Mu8. Nucleic Acids Res. 18, 6725 (1990).
23. Chomet, P., Lisch, D., Hardeman, K. J., Chandler, V. L. \& Freeling, M. Identification of a regulatory transposon that controls the Mutator transposable element system in maize. Genetics 129, 261-270 (1991).
24. Schnable, P. S., Peterson, P. A. \& Saedler, H. The bz-rcy allele of the Cy transposable element system of Zea mays contains a Mu-like element insertion. Mol. Gen. Genet. 217, 459-463 (1989).
25. Hershberger, R. J., Warren, C. A. \& Walbot, V. Mutator activity in maize correlates with the presence and expression of the Mu transposable element Mu9. Proc. Natl. Acad. Sci. U. S. A. 88, 10198-10202 (1991).
26. Dietrich, C. R. et al. Maize Mu transposons are targeted to the 5 ' untranslated region of the gl8 gene and sequences flanking Mu target-site duplications exhibit nonrandom nucleotide composition throughout the genome. Genetics 160, 697-716 (2002).
27. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792-1797 (2004).
28. Benito, M. I. \& Walbot, V. Characterization of the maize Mutator transposable element MURA transposase as a DNA-binding protein. Mol. Cell. Biol. 17, 5165-5175 (1997).

## Supplementary tables:

Supplementary Table 1. Gap number and sizes in maize genomes

|  | \# gaps (>10Ns) | Mean gap size | Total gap length |
| :---: | ---: | ---: | ---: |
| B73 | 2520 | 12196 | 30732868 |
| W22 | 68123 | 596 | 40626859 |
| PH207 | 362647 | 1219 | 442114873 |

Supplementary Table 2. Gene content variation in B73 and W22 relative to sorghum.

| B73 as query | Number of genes | \% of genes |
| :--- | ---: | ---: |
| Number of annotated nuclear genes <br> in study | 38254 |  |
| Present in W22 and Sorghum | 23072 | 60.3 |
| Present in W22 but not in Sorghum | 7861 | 20.5 |
| Present in Sorghum but not W22 | 881 | 2.3 |
| Not in W22 or Sorghum | 6440 | 16.8 |
|  |  |  |
| W22 as query |  |  |
| Number of annotated nuclear genes <br> in study | 40667 |  |
| Present in B73 and Sorghum | 24784 | 60.9 |
| Present in B73 but not in Sorghum | 6099 | 15.0 |
| Present in Sorghum but not B73 | 1412 | 3.5 |
| Not in B73 or Sorghum | 8372 | 20.6 |

Supplementary Table 3. The number of locally duplicated genes by the number of intervening genes. The number of genes determined to be local duplicates when the number of intervening genes varies from zero (tandem duplicates) to a maximum of 20.

|  | \# of Locally Duplicated Genes in |  |
| ---: | ---: | ---: |
| \# of Intervening Genes | W22 | B73 |
| 0 | 3,405 | 3,690 |
| 1 | 1,863 | 1,821 |
| 2 | 1,360 | 1,178 |
| 3 | 1,012 | 766 |
| 4 | 743 | 574 |
| 5 | 552 | 491 |
| $6-10$ | 1,017 | 765 |
| $11-20$ | 880 | 546 |
| Total | 6,034 | 5,768 |

Supplementary Table 4. Number of local multiplication clusters in B73 and W22 genomes. The distribution of local multiplication clusters was classified by the number of gene copies in each cluster. The distribution was determined allowing for a maximum of 20 intervening genes.

| \# of Copies | \# of Clusters |  |
| :---: | ---: | ---: |
|  | W22 | B73 |
| 2 | 1,706 | 1,683 |
| 3 | 344 | 342 |
| 4 | 130 | 119 |
| 5 | 62 | 61 |
| $6-10$ | 74 | 60 |
| $>10$ | 13 | 12 |
| Total | 2,329 | 2,277 |

Supplementary Table 5. Number of shared and unique tandem duplications between W22 and B73. Among the tandem duplications (two copies, zero intervening genes), the number that are shared between the two inbreds and unique to each inbred. The column descriptions are Head to head $(\mathrm{H}-\mathrm{H})$, Head to tail (H-T), Tail to Tail (T-T) and the total duplications for each row.

|  | \# of Tandem Duplications |  |  |  |
| :---: | ---: | ---: | ---: | ---: |
|  | H-H | H-T | T-T | Total |
| Unique to W22 | 215 | 862 | 184 | 1,261 |
| Shared (W22) | 90 | 510 | 78 | 678 |
| Shared (B73) | 85 | 511 | 82 |  |
| Unique to B73 | 242 | 925 | 227 | 1,394 |

Supplementary Table 6: Genome-wide alternative splicing in W22v2 (AltA: alternative acceptor; AltD: alternative donor; AltTE: alternate exon; ExonS: exon skip; IntronR: intron retention).

| AStype | Number of AS Genes | Number of AS Isoforms | Number of AS Events |
| :---: | ---: | ---: | ---: |
| AltA | 6,499 | 29,312 | 12,377 |
| AltD | 4,893 | 21,869 | 8,535 |
| AltTE | 3,082 | 12,773 | 5,033 |
| ExonS | 3,420 | 8,114 | 4,713 |
| IntronR | 10,078 | 27,258 | 29,939 |
| Total events | 13,591 | 58,279 | 60,597 |

Supplementary Table 7. Numbers of Mu-element TIRs in Group 1 through Group 7 identified in W22 and B73 (TIR, Terminal Inverted Repeat).

| Mu-TIR Group | B73 | W22 |
| :--- | ---: | ---: |
| Group1 | 96 | 99 |
| Group 2 | 89 | 81 |
| Group 3 | 90 | 94 |
| Group 4 | 95 | 106 |
| Group 5 | 63 | 48 |
| Group 6 | 38 | 34 |
| Group 7 | 10 | 12 |
| Total | 481 | 474 |

Supplementary Table 8. Mu element TIRs in W22 and B73 (v3) by chromosome (TIR, Terminal Inverted Repeat).

| Chromosome | Intact Element |  | Orphan TIRs |  | Tandem TIRs |  | Total TIRs |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | W22 | B73 | W22 | B73 | W22 | B73 | W22 | B73 |
| Chr 1 | 28 | 27 | 6 | 6 | 2 | 3 | 64 | 65 |
| Chr 2 | 26 | 29 | 18 | 6 | 2 | 3 | 72 | 67 |
| Chr 3 | 24 | 20 | 2 | 3 | 1 | 1 | 51 | 44 |
| Chr 4 | 26 | 23 | 2 | 4 | 0 | 1 | 54 | 51 |
| Chr 5 | 26 | 38 | 4 | 4 | 0 | 0 | 56 | 80 |
| Chr 6 | 9 | 14 | 5 | 8 | 10* | 1 | 33 | 37 |
| Chr 7 | 8 | 11 | 3 | 2 | 0 | 0 | 19 | 24 |
| Chr 8 | 22 | 22 | 9 | 6 | 0 | 0 | 53 | 50 |
| Chr 9 | 14 | 9 | 5 | 6 | 0 | 0 | 33 | 24 |
| Chr 10 | 17 | 13 | 3 | 5 | 2 | 0 | 39 | 31 |
| Chr Unk | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 8 |
| Total | 200 | 210 | 56 |  | 17 | 9 | 474 | 481 |

* One region on chromosome 6 of W22 contains an array of 10 tandem duplications of a single TIR (scored as one orphan and 9 tandem TIRs).

Supplementary Table 9. TIR families with greater than 10 copies in W22

| Family name | Superfamily | \# copies in W22 genome | Order in <br> Figure 4A |
| :---: | :---: | :---: | :---: |
| DTH13942 | DTH | 21 | 1 |
| DTH10730 | DTH | 124 | 2 |
| DTH11101 | DTH | 169 | 3 |
| DTH11209 | DTH | 74 | 4 |
| DTH15158 | DTH | 189 | 5 |
| DTH12258 | DTH | 40 | 6 |
| DTT10101 | DTT | 94 | 7 |
| DTH11270 | DTH | 254 | 8 |
| DTH11374 | DTH | 1514 | 9 |
| DTH11602 | DTH | 84 | 10 |
| DTH10268 | DTH | 122 | 11 |
| DTH10107 | DTH | 96 | 12 |
| DTH12507 | DTH | 35 | 13 |
| DTT10927 | DTT | 34 | 14 |
| DTH12298 | DTH | 67 | 15 |
| DTH12718 | DTH | 23 | 16 |
| DTT14784 | DTT | 20 | 17 |
| DTH12996 | DTH | 234 | 18 |
| DTH11238 | DTH | 38 | 19 |
| DTH16100 | DTH | 26 | 20 |
| DTH12997 | DTH | 107 | 21 |
| DTH16329 | DTH | 154 | 22 |
| DTH11541 | DTH | 139 | 23 |
| DTH10775 | DTH | 176 | 24 |
| DTH12973 | DTH | 24 | 25 |
| DTH10445 | DTH | 149 | 26 |
| DTH16443 | DTH | 34 | 27 |
| DTH13117 | DTH | 22 | 28 |
| DTH12864 | DTH | 31 | 29 |
| DTH16563 | DTH | 30 | 30 |
| DTT15264 | DTT | 25 | 31 |
| DTH13439 | DTH | 78 | 32 |
| DTH10818 | DTH | 44 | 33 |
| DTH10194 | DTH | 151 | 34 |
| DTH10187 | DTH | 58 | 35 |
| DTH10176 | DTH | 22 | 36 |
| DTH13854 | DTH | 58 | 37 |
| DTH16233 | DTH | 56 | 38 |
| DTH16174 | DTH | 25 | 39 |
| DTH13110 | DTH | 240 | 40 |


| DTH15132 | DTH | 39 | 41 |
| :---: | :---: | :---: | :---: |
| DTH13583 | DTH | 66 | 42 |
| DTH10856 | DTH | 592 | 43 |
| DTH10855 | DTH | 134 | 44 |
| DTA00256 | DTA | 24 | 45 |
| DTC00118 | DTC | 27 | 46 |
| DTH13261 | DTH | 23 | 47 |
| DTH10573 | DTH | 125 | 48 |
| DTH10113 | DTH | 21 | 49 |
| DTA00306 | DTA | 24 | 50 |
| DTH10239 | DTH | 23 | 51 |
| DTH11614 | DTH | 21 | 52 |
| DTA00295 | DTA | 36 | 53 |
| DTH10240 | DTH | 59 | 54 |
| DTH14736 | DTH | 44 | 55 |
| DTH10672 | DTH | 100 | 56 |
| DTH11388 | DTH | 36 | 57 |
| DTA00180 | DTA | 20 | 58 |
| DTT10062 | DTT | 45 | 59 |
| DTA00145 | DTA | 63 | 60 |
| DTA00114 | DTA | 22 | 61 |
| DTH11594 | DTH | 76 | 62 |
| DTT10089 | DTT | 52 | 63 |
| DTA00229 | DTA | 34 | 64 |
| DTA00234 | DTA | 255 | 65 |
| DTH10637 | DTH | 82 | 66 |
| DTH14738 | DTH | 36 | 67 |
| DTA00291 | DTA | 21 | 68 |
| DTH11674 | DTH | 21 | 69 |
| DTA00359 | DTA | 35 | 70 |
| DTA00100 | DTA | 23 | 71 |
| DTA00149 | DTA | 32 | 72 |
| DTC00030 | DTC | 58 | 73 |
| DTA00199 | DTA | 23 | 74 |
| DTH12306 | DTH | 39 | 75 |
| DTH10047 | DTH | 24 | 76 |
| DTH00410 | DTH | 45 | 77 |
| DTH00378 | DTH | 21 | 78 |
| DTT11073 | DTT | 41 | 79 |
| DTH00429 | DTH | 80 | 80 |
| DTH00058 | DTH | 136 | 81 |
| DTM00796 | DTM | 77 | 82 |
| DTM00473 | DTM | 188 | 83 |
| DTH00129 | DTH | 23 | 84 |


| DTA00294 | DTA | 24 | 85 |
| :---: | :---: | :---: | :---: |
| DTH00194 | DTH | 76 | 86 |
| DTH00437 | DTH | 197 | 87 |
| DTM01654 | DTM | 20 | 88 |
| DTH12389 | DTH | 22 | 89 |
| DTH00233 | DTH | 28 | 90 |
| DTA00200 | DTA | 34 | 91 |
| DTM00555 | DTM | 29 | 92 |
| DTT11230 | DTT | 22 | 93 |
| DTT11335 | DTT | 27 | 94 |
| DTH00127 | DTH | 22 | 95 |
| DTH00160 | DTH | 48 | 96 |
| DTM00299 | DTM | 72 | 97 |
| DTT11056 | DTT | 182 | 98 |
| DTH00163 | DTH | 48 | 99 |
| DTH11715 | DTH | 32 | 100 |
| DTM00257 | DTM | 272 | 101 |
| DTH00118 | DTH | 97 | 102 |
| DTA00111 | DTA | 56 | 103 |
| DTA00267 | DTA | 49 | 104 |
| DTH00051 | DTH | 76 | 105 |
| DTA00126 | DTA | 29 | 106 |
| DTH00434 | DTH | 24 | 107 |
| DTM00266 | DTM | 33 | 108 |
| DTH12490 | DTH | 34 | 109 |
| DTH00276 | DTH | 38 | 110 |
| DTH00458 | DTH | 114 | 111 |
| DTH00090 | DTH | 20 | 112 |
| DTH00460 | DTH | 26 | 113 |
| DTH00249 | DTH | 24 | 114 |
| DTH15359 | DTH | 57 | 115 |
| DTH00102 | DTH | 129 | 116 |
| DTH10388 | DTH | 37 | 117 |
| DTH00409 | DTH | 94 | 118 |
| DTH00489 | DTH | 21 | 119 |
| DTA00323 | DTA | 20 | 120 |
| DTA00242 | DTA | 20 | 121 |
| DTA00364 | DTA | 24 | 122 |
| DTH00412 | DTH | 26 | 123 |
| DTT10009 | DTT | 36 | 124 |
| DTA00383 | DTA | 30 | 125 |
| DTA00166 | DTA | 24 | 126 |
| DTA00140 | DTA | 34 | 127 |
| DTA00139 | DTA | 80 | 128 |


| DTA00322 | DTA | 84 | 129 |
| :--- | :--- | :--- | :--- |
| DTA00231 | DTA | 78 | 130 |
| DTA00110 | DTA | 22 | 131 |
| DTA00373 | DTA | 75 | 132 |
| DTA12512 | DTA | 35 | 133 |
| DTA00040 | DTA | 379 | 134 |
| DTC12155 | DTC | 34 | 135 |
| DTH13200 | DTH | 30 | 136 |
| DTA00346 | DTA | 30 | 137 |
| DTA00240 | DTA | 22 | 138 |
| DTT11465 | DTT | 54 | 139 |
| DTH11592 | DTH | 31 | 140 |
| DTA00177 | DTA | 64 | 141 |
| DTA00098 | DTA | 160 | 142 |
| DTA00334 | DTA | 83 | 143 |
| DTH11615 | DTH | 76 | 144 |
| DTA00263 | DTA | 39 | 145 |
| DTA00179 | DTA | 42 | 146 |
| DTA00151 | DTA | 31 | 147 |
| DTA00217 | DTA | 110 | 148 |
| DTA00133 | DTA | 26 | 149 |
| DTA00327 | DTA | 28 | 150 |
| DTA00268 | DTA | 35 | 151 |
| DTA00104 | DTA | 32 | 152 |
| DTA00307 | DTA | 56 | 153 |
| DTM00800 | DTM | 37 | 154 |
| DTA00204 | DTA | 42 | 155 |
| DTA00313 | DTA | 20 | 156 |
| DTA00155 | DTA | 42 | 157 |
| DTA00333 | DTA | 20 | 158 |
| DTA00178 | DTA | 28 | 159 |
| DTA00300 | DTA | 53 | 160 |
| DTA00261 | DTA | 49 | 161 |
| DTA00208 | DTA | 54 | 162 |
| DTH12617 | DTH | 79 | 172 |
| DTH10658 | DTH | 26 | 163 |
| DTA00368 | DTA | 47 | 164 |
| DTH12502 | DTH | 31 | 165 |
| DTH10440 | DTH | 21 | 166 |
| DTA00073 | DTA | 28 | 167 |
| DTH10328 | DTH | 35 | 168 |
| DTH16801 | DTH | 33 | 169 |
| DTH | 34 | 170 |  |
| DTH | DTH | 34 | 171 |


| DTA00252 | DTA | 40 | 173 |
| :--- | :--- | :--- | :--- |
| DTA00188 | DTA | 23 | 174 |
| DTM00743 | DTM | 77 | 175 |
| DTH00327 | DTH | 26 | 176 |
| DTA00163 | DTA | 72 | 177 |
| DTA00117 | DTA | 89 | 178 |
| DTM00460 | DTM | 30 | 179 |
| DTH10310 | DTH | 59 | 180 |
| DTA00106 | DTA | 44 | 181 |
| DTA00165 | DTA | 21 | 182 |
| DTA13185 | DTA | 71 | 183 |
| DTA00156 | DTA | 45 | 184 |
| DTA00153 | DTA | 33 | 185 |
| DTH12584 | DTH | 38 | 186 |
| DTM00268 | DTM | 83 | 187 |
| DTC00122 | DTC | 73 | 188 |
| DTA00283 | DTA | 234 | 189 |
| DTA00169 | DTA | 191 | 190 |
| DTC00119 | DTC | 99 | 191 |


[^0]:    ${ }^{1}$ Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, USA. ${ }^{2}$ USDA-ARS, Corn Insects and Crop Genetics Research Unit and Iowa State University, Department of Computer Science, lowa State University, Ames, IA, USA. ${ }^{3}$ Boyce Thompson Institute, Ithaca, NY, USA. ${ }^{4}$ Horticultural Sciences Department, University of Florida, Gainesville, FL, USA. ${ }^{5}$ NRGene Ltd, Ness Ziona, Israel. ${ }^{6}$ Department of Biology and the UF Genetics Institute, University of Florida, Cancer \& Genetics Research Complex, Gainesville, FL, USA. ${ }^{7}$ Department of Biological Science, The Florida State University, Tallahassee, FL, USA. ${ }^{8}$ USDA-ARS, Holley Center for Agriculture and Health, Ithaca, NY, USA. ${ }^{9}$ Institute for Genomic Diversity, Biotechnology Building, Cornell University, Ithaca, NY, USA. ${ }^{10}$ Cold Spring Harbor Laboratory, Cold Springs Harbor, NY, USA. ${ }^{11}$ Department of Plant Biology, University of Georgia, Athens, GA, USA. ${ }^{12}$ Department of Plant Biology, Rutgers University, New Brunswick, NJ, USA. ${ }^{13}$ Waksman Institute, Rutgers University, Piscataway, NJ, USA. ${ }^{14}$ Department of Biology, Montclair State University, Montclair, NJ, USA. ${ }^{15}$ USDA-ARS Chemistry Research Unit, Gainesville, FL, USA. ${ }^{16}$ Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany. ${ }^{17}$ Metabologenomics, Inc., Tsuruoka, Yamagata, Japan. ${ }^{18} \mathrm{CAS}$-JIC Centre of Excellence for Plant and Microbial Science (CEPAMS), Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China. ${ }^{19}$ The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China. ${ }^{20}$ Donald Danforth Plant Science Center, St. Louis, MO, USA. ${ }^{21}$ Bionano Genomics, San Diego, CA, USA. ${ }^{22}$ Department of Plant Sciences and Center for Population Biology, University of California, Davis, Davis, California, USA. ${ }^{23}$ Center for Genomics and Personalized Medicine, The Florida State University, Tallahassee, FL, USA. ${ }^{24}$ Department of Genetics, Development and Cell Biology, lowa State University, Ames, IA, USA. ${ }^{25}$ USDA-ARS, NEA Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY, USA. ${ }^{26}$ Present address: College of Agronomic Sciences State Key Laboratory of Crop Biology, Shandong Agricultural University, Shandong, China. *e-mail: brutnell@gmail.com

