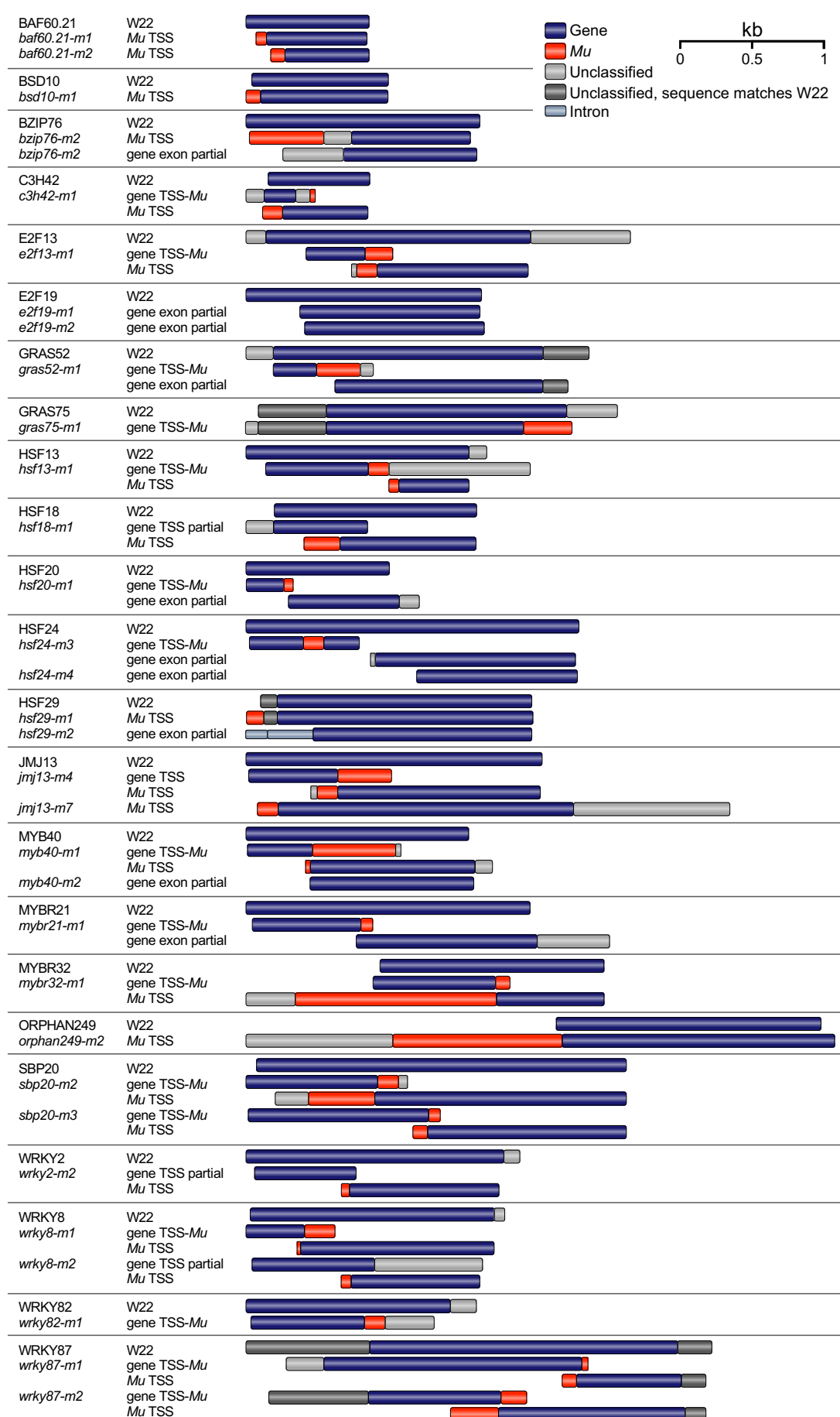
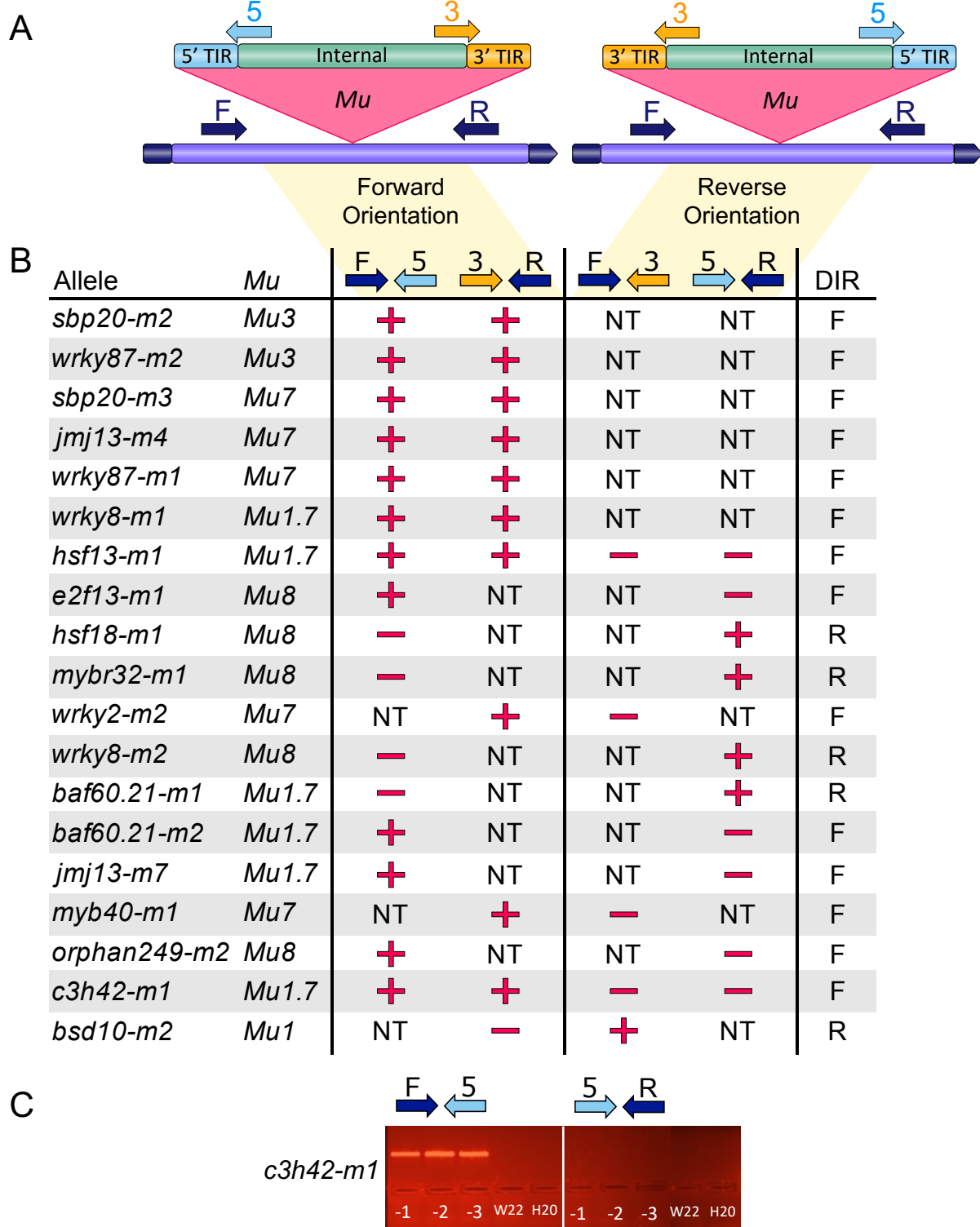


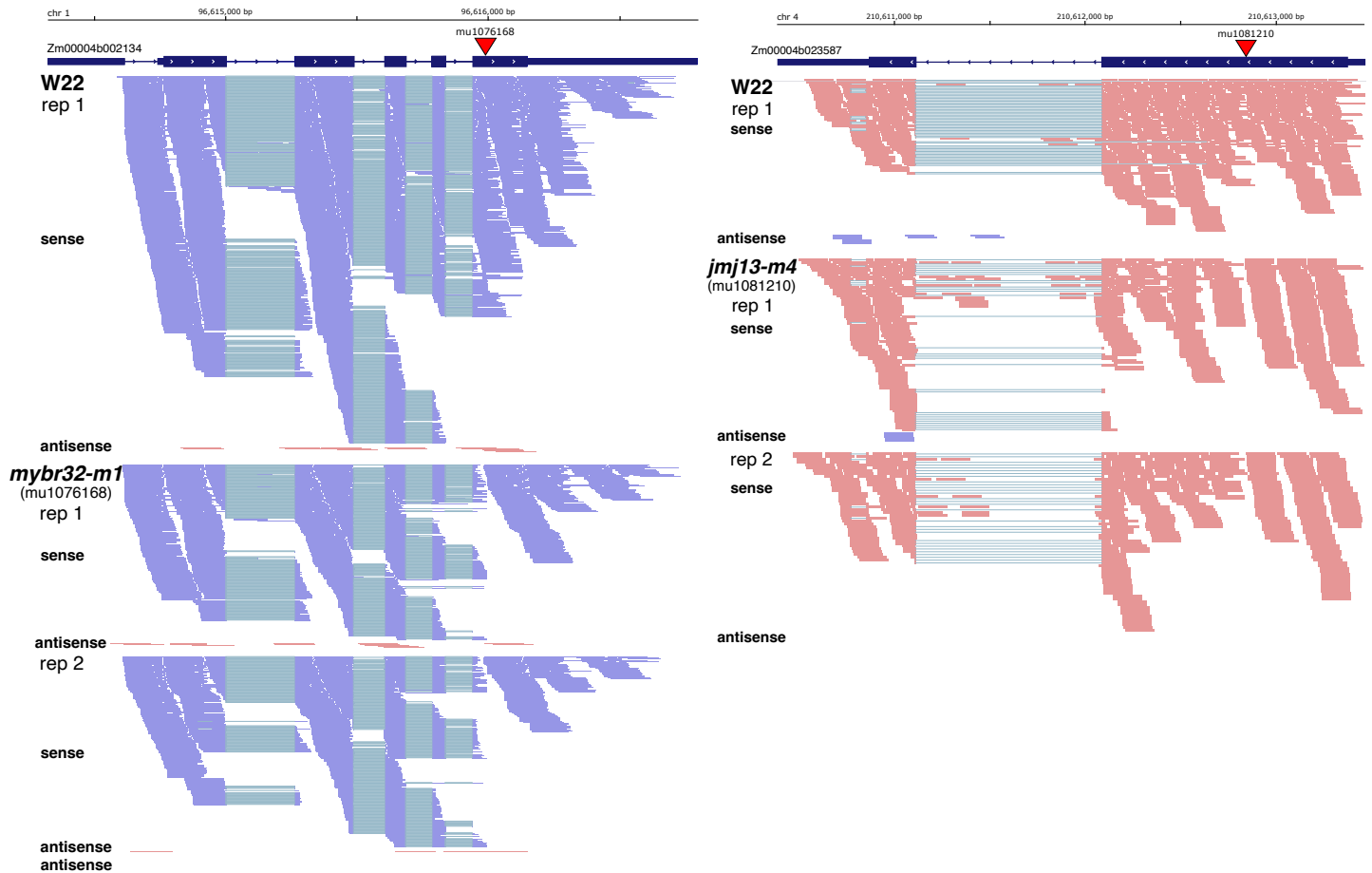
**Figure S1. Visualization of wild-type W22 and mutant allele RNA-seq read coverage for three genes.** IGV was utilized to visualize the coverage of RNA-seq reads for one biological replicate of each mutant allele for three genes that each have two mutant alleles; *SBP20* (Zm00004b024383), *WRKY87* (Zm00004b023521) and *BAF60.21* (Zm00004b012791). The mutant alleles for: *SBP20* (green), *sbp20-m2* (mu1084360) and *sbp20-m3* (mu1091327); *WRKY87* (orange), *wrky87-m1* (mu1067257) and *wrky87-m2* (mu1091217); *BAF60.21* (blue), *baf60.21-m1* (mu1034781) and *baf60.21-m2* (mu1092086) all show reduced coverage in the regions flanking their respective annotated *Mu* insertions (triangles) in the mutant samples compared to W22 wild-type (black).



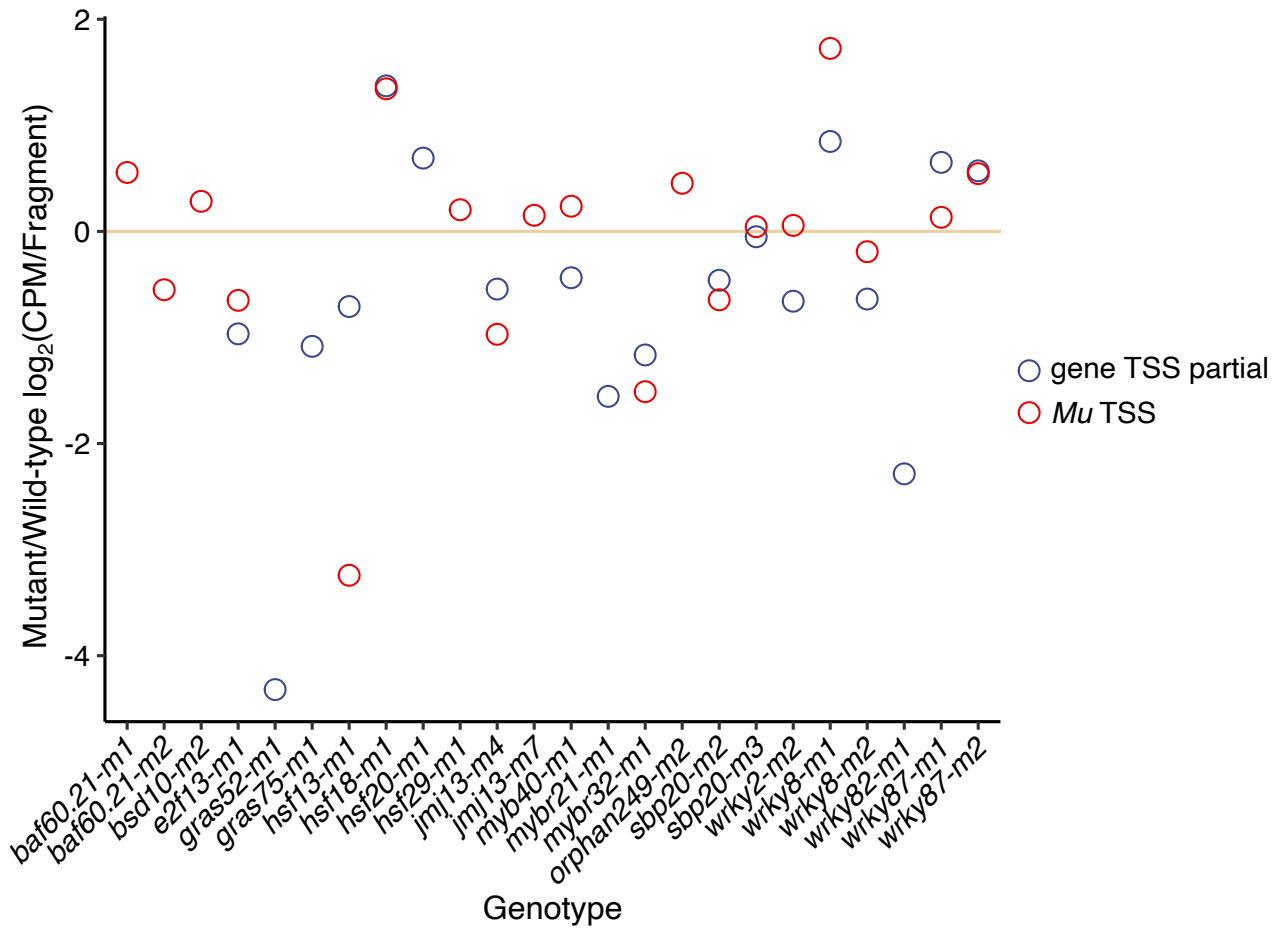
**Figure S2. De novo assembly transcript structures of 33 mutant alleles and wild-type W22 control.** Schematic of transcripts assembled from the RNA-seq data generated for each wild-type W22 gene and corresponding mutant allele(s). All transcripts are shown to scale by sequence length (kilobase scale included). Mutant allele transcripts are positioned based on alignments to the W22 transcript assembly for each gene. Transcript sequences are colored by sequence identity to the annotated gene cDNA (navy), *Mu* (red), neither the annotated gene cDNA or *Mu* (unclassified, gray), or intron (blue). Unclassified sequence that is shared between W22 and mutant allele transcript assemblies is colored dark gray.



**Figure S3. Determination of *Mu* element identity and orientation by PCR using *Mu* element specific primers. A)** Schematic of genomic DNA primer design for identifying the forward or reverse orientation of *Mu*. Gene specific primers (F = Forward, R = Reverse: navy) and predicted *Mu* element primers with specificity to either the 5' TIR (5: light blue) or the 3' TIR (3: yellow) of the predicted *Mu* element were used to complete PCR for each mutant allele. The presence of PCR amplicons from F-5 and R-3 indicate orientation of the *Mu* element in the forward direction (5'TIR to 3'TIR) and the presence of PCR amplicons from F-3 and R-5 indicate orientation of the *Mu* element in the reverse direction (3'TIR to 5'TIR). **B)** The PCR results of 19 mutant alleles are depicted in a table by amplicon presence (+), absence (-), or not tested (NT). For the alleles with predictions of *Mu* element identity and orientation we only tested the predicted *Mu* element. For other alleles we tested a variety of primers and both potential orientations and only show the results for the *Mu* element that provided amplification. *bsd10-m2* may be either *Mu1* or *Mu1.7*. **C)** Gel image of PCR amplicons for *c3h42-m1* biological replicates (-1, -2, -3), F-5 presence (+) and R-5 absence (-), and W22 wild-type and water as controls.



**Figure S4. Analysis of RNA-seq read orientation between wild-type W22 and the mutant allele for two genes.** IGV was used to visualize the orientation of RNA-seq reads mapped to the wild-type gene with the annotated *Mu* insertion (red triangle) in one control W22 biological replicate and two mutant allele biological replicates for two genes: *MYBR32* (Zm00004b002134) and *JMJ13* (Zm00004b023587). The RNA-seq data for both mutant and wild-type alleles was generated from directional/stranded libraries. Reads were grouped and colored by the read strand of the first read in each pair of paired-end reads (IGV “first-of-pair strand”) with the read color matching the direction of the transcript orientation; purple for the negative or reverse complement DNA strand and pink for the positive (5' to 3') DNA strand. Sense refers to the coding or nontemplate strand of DNA and antisense refers to the template strand of DNA. No antisense reads were observed for biological replicate 2 or 3 (not shown) of *jmj13-m4*. Read orientation was examined to identify the potential for bi-directional transcripts originating from the *Mu* promoter in the mutant alleles.



**Figure S5. Ratios of mutant to wild-type transcript abundance.** A scatter plot of the log<sub>2</sub> normalized ratio of mutant to wild-type transcript abundance (CPM/fragment) is plotted for each mutant allele and the corresponding mutant transcript, gene TSS partial (blue circles) and *Mu* TSS (red circles). Mutant transcripts with expression levels that vary relative to wild-type transcripts are plotted relative to zero with positive values for higher transcript abundance and negative values for lower transcript abundance.