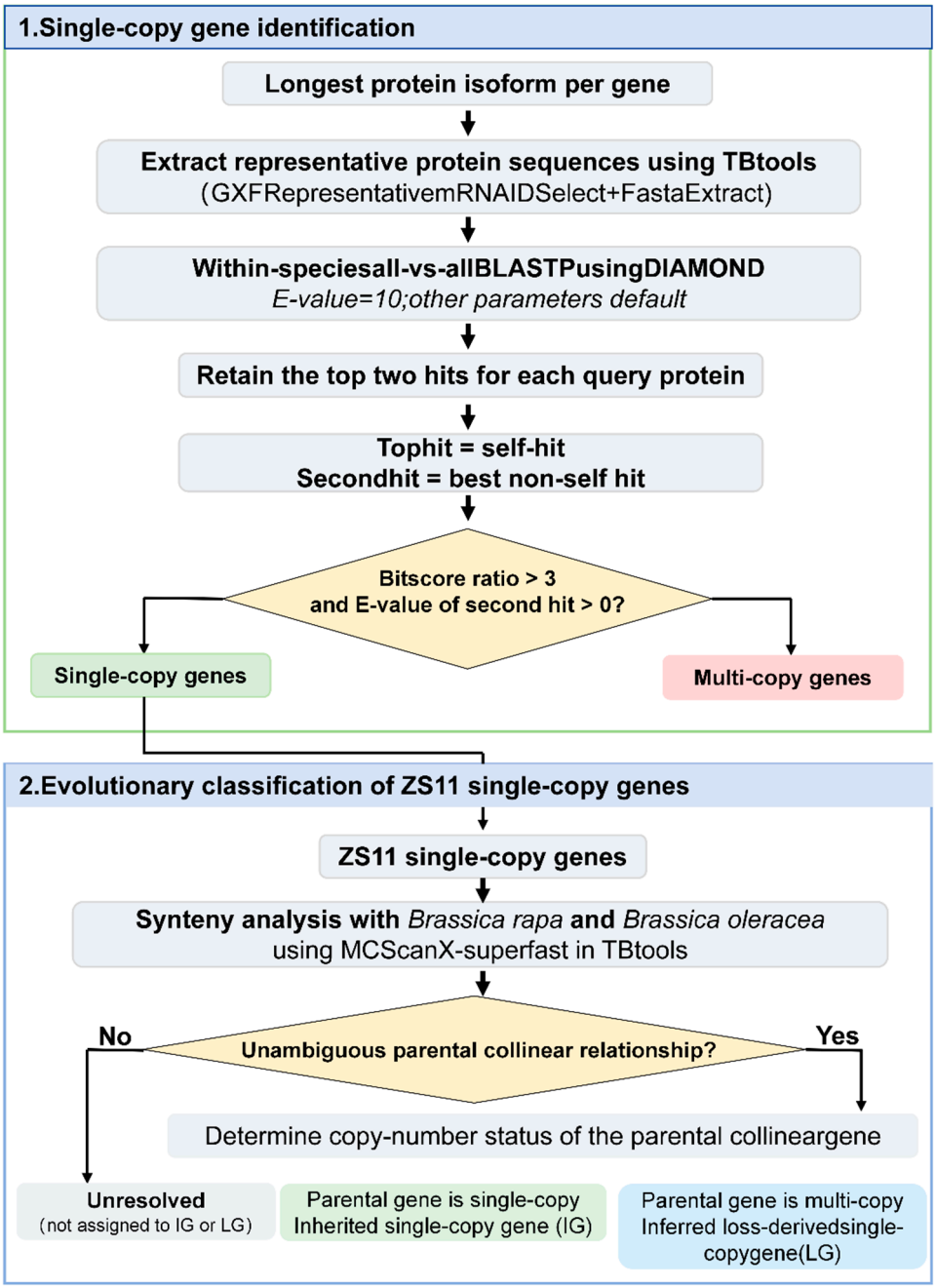


IG,inherited single-copy gene;LG,inferred loss-derived single-copy gene.

Figure S1. Workflow for single-copy gene identification and evolutionary classification.

For genes with multiple transcripts, the transcript encoding the longest protein was selected as the representative transcript using TBtools, and the corresponding protein sequence was extracted for subsequent analysis. Representative protein sequences were subjected to within-species all-versus-all BLASTP analysis using DIAMOND, with the E-value parameter set to 10 and all other parameters left at their default settings. For each query protein, the two highest-scoring hits were retained. The highest-scoring hit was designated the self-hit, whereas the second-highest-scoring hit represented the best non-self hit. A protein was classified as a single-copy gene when the self-hit/second-hit bitscore ratio was greater than 3 and the E-value of the second-best hit was greater than 0; otherwise, it was classified as a multi-copy gene. For ZS11 single-copy genes, synteny analysis with *Brassica rapa* and *Brassica oleracea* was subsequently performed using MCScanX-

superfast in TBtools. Genes with unambiguous parental collinear relationships were further classified according to the copy-number status of the corresponding parental collinear gene. Genes whose parental collinear genes were single-copy were classified as inherited single-copy genes (IGs), whereas those whose parental collinear genes were multi-copy were classified as inferred loss-derived single-copy genes (LGs). Genes lacking unambiguous parental collinearity were left unresolved and were not assigned to either category.

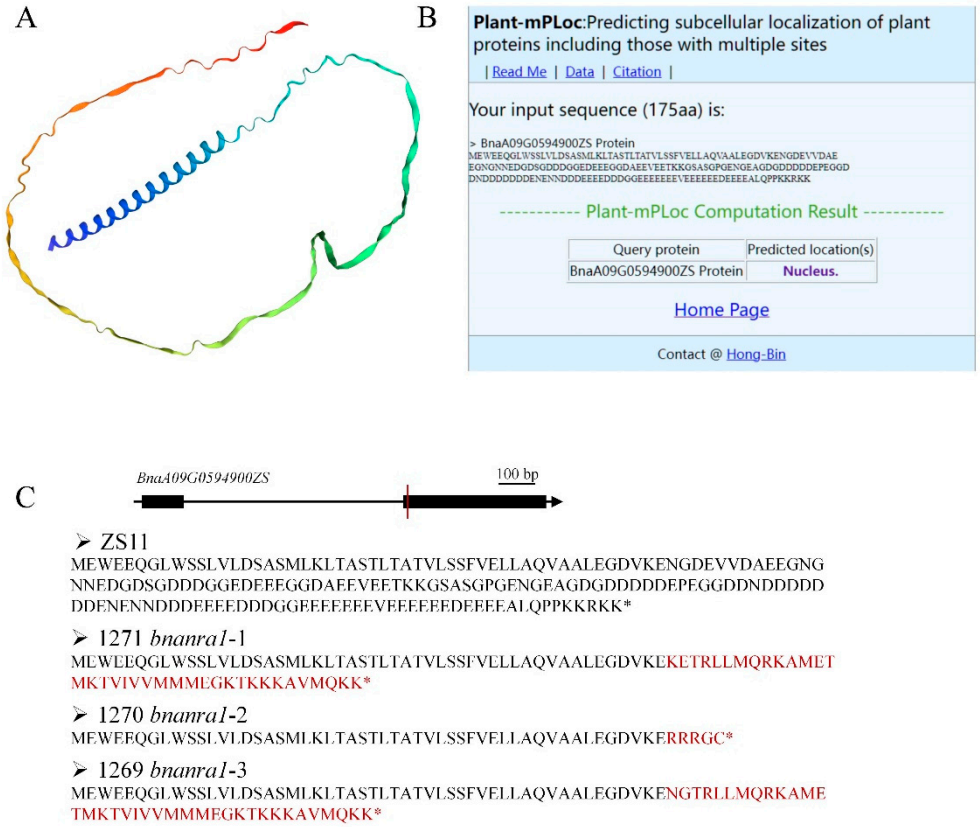


Figure S2. Predicted structural features of *BnaNRA1* and sequence characterization of the *bnanra1* edited alleles.

(A) Predicted three-dimensional structure of the BnaNRA1 protein. (B) Subcellular localization prediction of BnaNRA1 using Plant-mPLoc, indicating a predicted nuclear localization. (C) Gene structure of BnaNRA1 and predicted amino acid sequences of ZS11 and three independent CRISPR/Cas9-edited alleles, *bnanra1*-1 (1271), *bnanra1*-2 (1270), and *bnanra1*-3 (1269). Black boxes represent exons, the connecting line represents the intron, and the red vertical line indicates the CRISPR/Cas9 target site. Amino acid residues altered by frameshift mutations are shown in red, and asterisks indicate translation stop codons. The scale bar represents 100 bp.