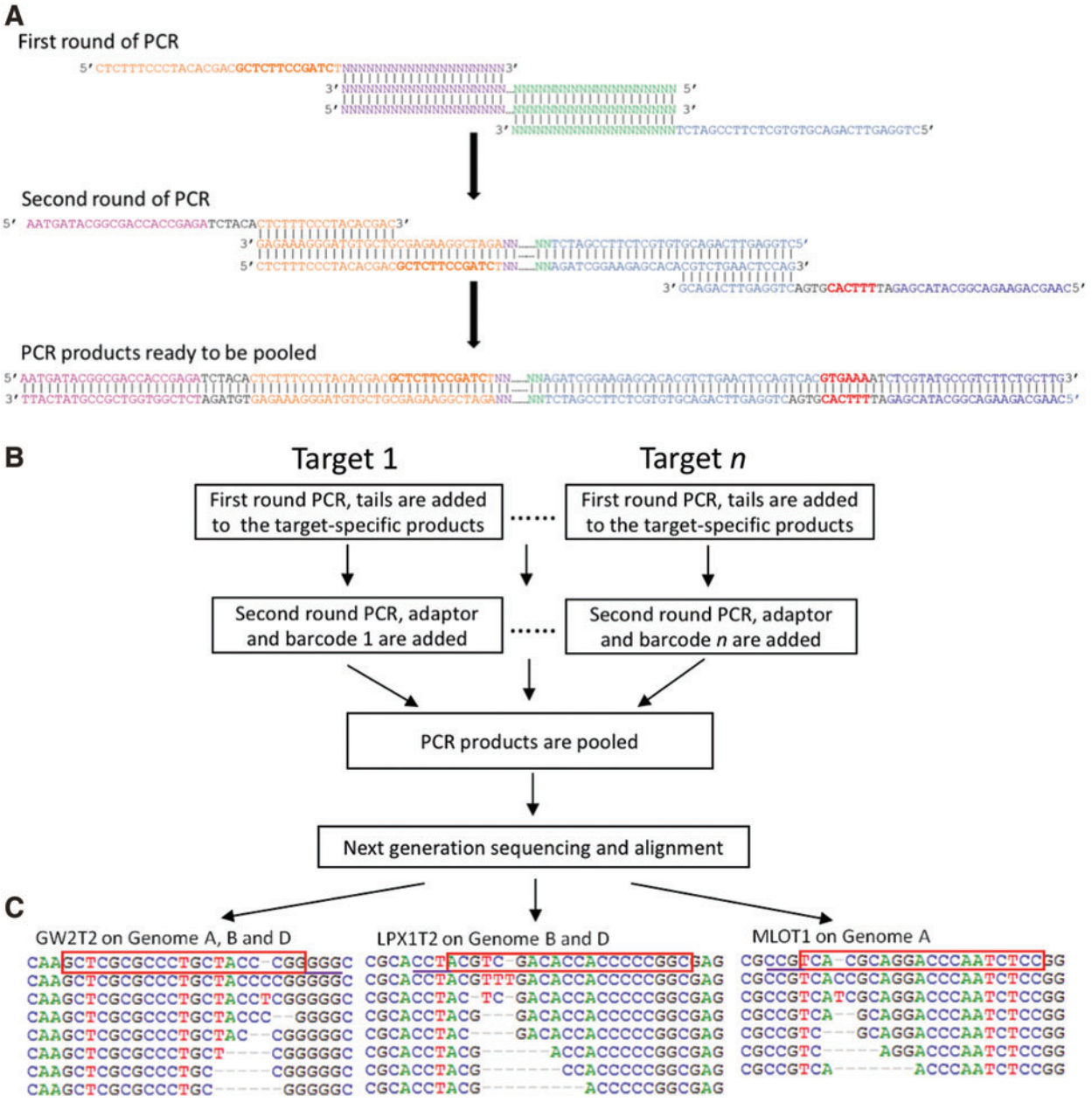

SUPPLEMENTARY FIG. S3. NGS-based workflow for estimating the CRISPR-Cas9 gene editing efficiency in the wheat protoplast and plant tissues. **(A)** Sequences of primers used to generate barcoded amplicons for multiplexed NGS. Two rounds of PCRs are used to add Illumina TruSeq adapters and multiplexing barcodes to target-specific PCR products. Target-specific sequences are shown as "Ns". As an example, the sequence of TruSeq barcode 19 is shown in red. **(B)** Workflow of multiplexed PCR amplicon library preparation for NGS. Multiple targets are amplified and barcoded in two rounds of PCR, pooled, and sequenced on the Illumina MiSeq instrument. **(C)** Alignment of NGS reads generated for the target regions in the *TaGW2*, *TaLpx-1*, and *TaMLO* genes. Illumina reads were aligned to the wild-type reference sequences. The target sequences are shown in the red rectangles; the PAM sequences are underlined.