

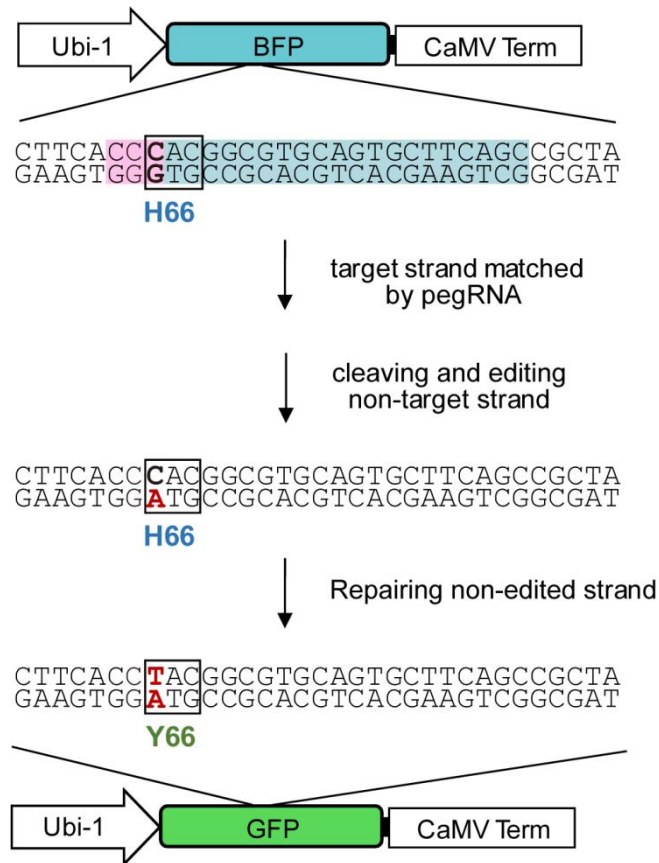
## Supplementary information

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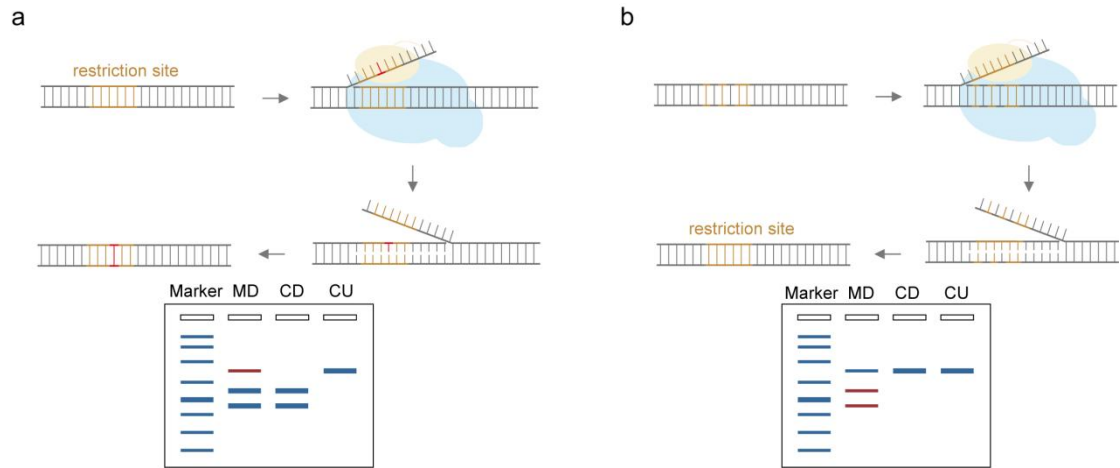
# Optimized prime editing in monocot plants using PlantPegDesigner and engineered plant prime editors (ePPEs)

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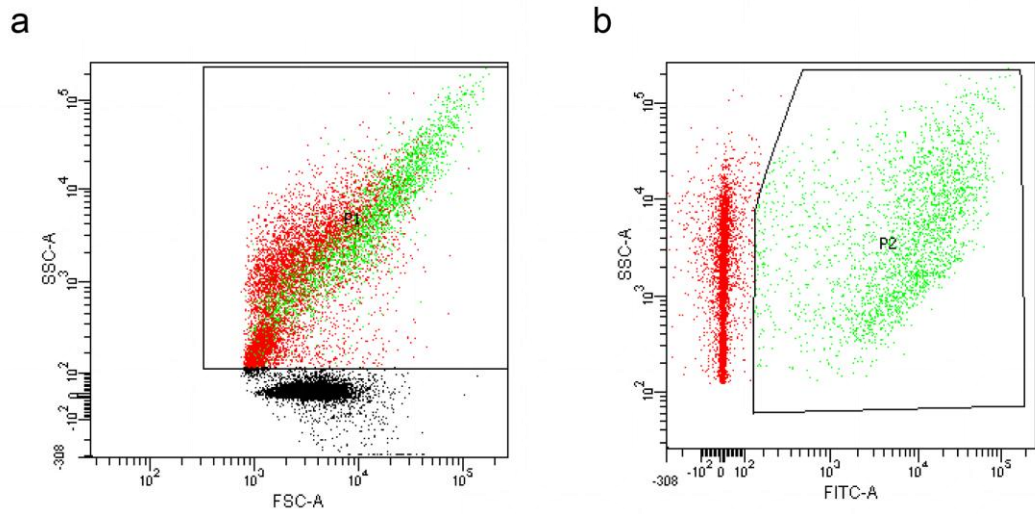
In the format provided by the authors and unedited



**Supplementary Figure 1** Schematic representation of the BFP-to-GFP reporter system for prime editing (corresponding to Box 2). The plant prime editors can change the BFP to GFP by changing CAC (histidine) to TAC (tyrosine). The target sequence and PAM motif of pegRNA are shown in blue and pink, respectively. The target sequence and PAM motif of pegRNA are shown in blue and pink, respectively. Figure adapted with permission from ref.<sup>5</sup>, Springer Nature America.



**Supplementary Figure 2** Schematic of the PCR/RE assay used to detect prime editing events. The yellowish and blue complex represent prime editors. The red base represent prime edited bases. The brown base represent bases in restriction site. (a) The amplicons amplified from genomic DNA of transgenic plants are digested with restriction enzymes that recognize the wild-type target sequences. Mutations introduced by PE are resistant to restriction enzyme digestion because of the loss of the restriction sites, and they result in an uncleaved band (colored in red) in agarose gels. (b) The amplicons amplified from genomic DNA of transgenic plants are digested with restriction enzymes that recognize the prime edited sequences. Prime edited sequence could be designed to create a new restriction site if possible. Mutations introduced by PE are digested by restriction enzyme digestion, and they result in a cleaved band (colored in red) in agarose gels. M, Marker; MD, Mutant digested; CD, Control digested; CU, Control undigested.



**Supplementary Figure 3** Representative flow cytometry and gating strategy to isolate protoplasts. All events are discriminated by size, granularity, and viability. (a) The first gate (P1) is drawn on a plot of side scatter-area (SSC-A) versus forward scatter-area (FSC-A), excluding a narrow strip of debris. (b) The second gate (P2) is drawn on a plot of side scatter-area (SSC-A) versus fluorescence of fluorescein isothiocyanate-area (FITC-A) to discern single cells.

**Supplementary Note 1. Complete sequences of the nCas9-ePPE in this study.** The sequences of Ubi promoter, nCas9, and M-MLV reverse transcriptase are highlighted in red, blue, and purple, respectively.

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**Supplementary Note 2. Complete sequences of the pH-ePPE in this study.** The sequences of Ubi promoter, nCas9, and M-MLV reverse transcriptase are highlighted in red, blue, and purple, respectively.

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**Supplementary Note 3. Sequence of genomic DNA containing OsCDC48T1 target site in this study.** The sequence of OsCDC48T1 target site, the sequence of first-round primers, and the sequence of second-round primers are highlighted in red, blue, and purple, respectively.

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