Supplementary Notes

Supplementary Note 1. The format of the input sequence

The input sequence of PlantPegDesigner contains both the reference sequence and the edited sequence. The designed edits are marked "(a/b)". "a" is the original sequence and "b" is the desired mutant sequence; either "a" or "b" may be null values representing insertions "(/b)" or deletions "(a/)", respectively (see examples of base conversions, insertions and deletions below). PlantPegDesigner also supports introducing multiple edits in one pegRNA (see examples of multiple edits below). Both uppercase and lowercase are accepted. We recommend that the input sequence contain at least 50 bp of upstream sequence and at least 50 bp of downstream sequence.

Example of a base substitution sequence (*OsALS-T1*, +2 A to G):

CTCGAGACTCCAGGGCCATACTTGTTGGATATCATCGTCCCGCACCAGGAG
CATGTGCTGCCTATGATCCCA(A/G)GTGGGGGCGCATTCAAGGACATGATCC
TGGATGGTGATGGCAGGACTGTGTA

Example of a sequence containing an insertion (*OsCDC48-T2*, +2 AAA insertion):

CTTCGCCCAGACTCTGCAGCAGTCTCGTGGGTTCGGCACCGAGTTTAGGTT
CGCTGACCAGCCAGCGTCTGGC(/AAA)GCCGGCGCCGCCGCTGACCCCTTC
GCATCCGCTGCCGCCGCAGCTGACGATGATGATT

Example of a sequence containing a deletion (*OsCDC48-T1*, +1–6 CTCCGG deletion):

CTTCTCAGCTTCTTCAAATGCCTTCCTGAGATTACTCTCACTTTCTCCTGCTA
GCTTTGACATAAT(CTCCGG/)GCCATTAATCAGAAAGAAGAAAGCACCTGTT
TCATTAGCAACAGCTCTA

Example of a sequence containing multiple edits (*OsCDC48-T1*, +1 C to T & +6 G to T):

CTTCTCAGCTTCTTCAAATGCCTTCCTGAGATTACTCTCACTTTCTCCTGCTA
GCTTTGACATAAT(CTCCGG/TTCCGT)GCCATTAATCAGAAAGAAGAAAGCA
CCTGTTTCATTAGCAACAGCTCTA

Supplementary Note 2. Parameters and an output example.

PlantPegDesigner provides a variety of choices of parameters to meet the different needs of users:

“PAM sequence” (default to NGG PAM), “Cut distance to PAM” (default to -3 position) and “Spacer length” (default to 20 nt): These three parameters depend on the type of Cas protein used, default to SpCas9.

“Spacer GC content” (default to 0%-100%): It has been reported that on-target GC content can influence the on-target editing activity of Cas9, so users can change this parameter if needed[1-3].

“Prime editing window” (default to +1 - +15): We define the nicked site as +1 and the NGG PAM as +4 to +6. Previous studies showed that prime editor can work efficiently in an editing window from +1 to +15 in plants[4-11], but longer editing window have also been reported[5-8,10]. The user can change these parameters.

“PBS length” (default to 7-16 bp) and “PBS GC content” (default to 0%-100%): The default values are based on previous reports[4-12].

“Recommended Tm of PBS sequence” (default to 30□): We strongly recommend the PBS Tm is set to 30□. If 30□ is unavailable, 32□ is recommended.

“Homologous RT template length” (default to 7-16 bp) and “Exclude first C in RT template” (default to true): The default values are based on previous results[12]. Caution: The Homologous RT template length is the length from the desired edits to the 3’ terminus, not the length of the whole RT template.

“Tm-directed PBS length model” and “Dual-pegRNA model”: These two models are based on the experimental results to design efficient PBS lengths and dual-pegRNAs (default to turn on). If the two models are turned off, the Tm-directed PBS length and dual-pegRNA will not be recommended. Also, if the Tm-directed PBS length model is turned off, the reverse primer would not be recommended.

In the output page of PlantPegDesigner, all the available spacer-PAM sequences are ranked by the distance between the nCas9-induced nick site and the desired edits, and are marked as “No. X program”. For each program, all PBS and RT template sequences are reported. For the RT template, PlantPegDesigner recommends one sequence of

median length that does not begin with “C”, which may not be the optimal RT template[12]. PlantPegDesigner reports all the RT template sequences of varying length, so users can test more pegRNAs with different RT template lengths if higher editing efficiencies are needed.

Supplementary Note 3. Primer design and vector construction

PlantPegDesigner provides four types of vector for pegRNA construction, and users can also specify the vector backbone sequence. The pOsU3, pTaU3, and pTaU6 vectors are recommended for particle bombardment and protoplast transformation experiments. The pH-nCas9-PPE-V2 vector is recommended for particle bombardment or *Agrobacterium*-mediated monocotyledon transformation. We recommend using the one-step PCR strategy and Gibson assembly for vector construction[4], in which sgRNAs are amplified using primer sets containing the spacer sequences in the forward primer and the PBS+RT template sequences in the reverse primer, and are cloned into the BsaI and HindIII-linearized pOsU3, pTaU6 and pH-nCas9-PPE-V2 vectors, or Esp3I and NcoI-linearized pTaU3 vectors.

Supplementary Note 4. Design of pooled pegRNAs

PlantPegDesigner permits the design of up to 50 pooled pegRNAs with different input sequences. If more than 50 pegRNAs are needed, please contact us by E-mail. The output of the pooled design is shown in the output page, and all the recommended results can be directly downloaded. Both uppercase and lowercase are accepted. The format of the pooled input file is as below:

>Input sequence 1

CTCGAGACTCCAGGGCCATACTTGTTGGATATCATCGTCCCGCACCAGGAG

CATGTGCTGCCTATGATCCCA(A/G)GTGGGGGCGCATTCAAGGACATGATCC

TGGATGGTGATGGCAGGACTGTGTA

>Input sequence 2

CTTCGCCCAGACTCTGCAGCAGTCTCGTGGGTTCGGCACCGAGTTTAGGTT

CGCTGACCAGCCAGCGTCTGGC(/AAA)GCCGGCGCCGCCGCTGACCCCTTC
GCATCCGCTGCCGCCGCAGCTGACGATGATGATT

……

>Input sequence 50

TAGAGCTGTTGCTAATGAAACAGGTGCTTTCTTCTTTCTGATTAATGGC(CC
GGAG/AGGCA)ATTATGTCAAAGCTAGCAGGAGAAAGTGAGAGTAATCTCA
GGAAGGCATTTGAAGAAGCTGAGAAG

References

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