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Combined fluorescent seed selection and multiplex CRISPR/Cas9 assembly for fast generation of multiple Arabidopsis mutants

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摘要	Background Multiplex CRISPR-Cas9-based genome editing is an efficient method for targeted disruption of gene function in plants. Use of CRISPR-Cas9 has increased rapidly in recent years and is becoming a routine method for generating single and higher order Arabidopsis thaliana mutants. Low entry, reliable assembly of CRISPR/Cas9 vectors and efficient mutagenesis is necessary to enable a maximum of researchers to break through the genetic redundancy within plant multi-gene families and allow for a plethora of gene function studies that have been previously unachievable. It will also allow routine de novo generation of mutations in ever more complex genetic backgrounds that make introgression of pre-existing alleles highly cumbersome. Results To facilitate rapid and efficient use of CRISPR/Cas9 for Arabidopsis research, we developed a CRISPR/Cas9-based toolbox for generating mutations at multiple genomic loci, using two-color fluorescent seed selection. In our system, up-to eight gRNAs can be routinely introduced into a binary vector carrying either a FastRed, FastGreen or FastCyan fluorescent seed selection cassette. FastRed and FastGreen binary vectors can be co-transformed as a cocktail via floral dip to introduce sixteen gRNAs at the same time. The seeds can be screened either for red or green fluorescence, or for the presence of both colors. Importantly, in the second generation after transformation, Cas9 free plants are identified simply by screening the non-fluorescent seeds. Our collection of binary vectors allows to choose between two widely-used promoters to drive Cas enzymes, either the egg cell-specific (pEC1.2) from A. thaliana or the constitutive promoter from Petroselinum crispum (PcUBi4-2). Available enzymes are “classical” Cas9 codon-optimized for A. thaliana and a recently reported, intron-containing version of Cas9 codon-optimized for Zea mays, zCas9i. We observed the highest efficiency in producing knockout phenotypes by using intron-containing zCas9i driven under egg-cell specific pEC1.2 promoter. Finally, we introduced convenient restriction sites flanking promoter, Cas9 and fluorescent selection cassette in some of the T-DNA vectors, thus allowing straightforward swapping of all three elements for further adaptation and improvement of the system. Conclusion A rapid, simple and flexible CISPR/Cas9 cloning system was established that allows assembly of multi-guide RNA constructs in a robust and reproducible fashion, by avoiding generation of very big constructs. The system enables a flexible, fast and efficient screening of single or higher order A. thaliana mutants.
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