BRC Science Highlight
February 2022

CROPSR: An Automated Platform for Complex Genome-Wide CRISPR gRNA Design and Validation

Background/objective

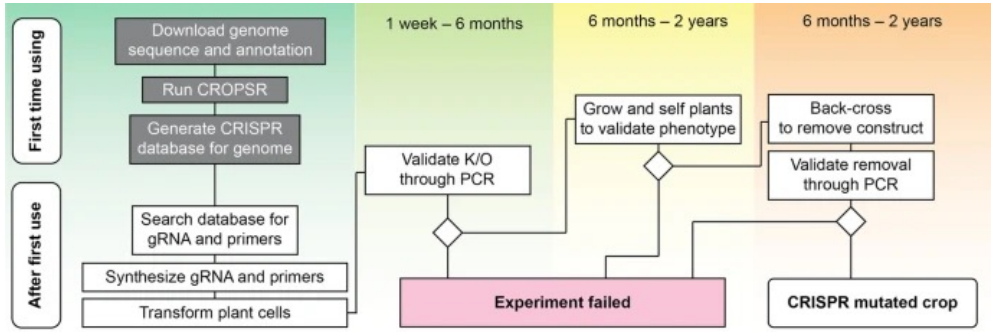
CRISPR/Cas9 technology has become an important tool to generate targeted, highly specific genome mutations. The technology has great potential for crop improvement, as crop genomes are tailored to optimize specific traits over generations of breeding. Many crops have highly complex and polyploid genomes, particularly those used for bioenergy or bioproducts. The majority of tools currently available for designing and evaluating gRNAs for CRISPR experiments were developed based on mammalian genomes that do not share the characteristics or design criteria for crop genomes. Here, we describe the development of CROPSR, an open-source tool for genome-wide design and evaluation of gRNA sequences for CRISPR experiments.

Approach

- CROPSR is a tool developed from the ground up as an open-source Python application to perform all steps required to design guide and primer sequences for genome editing, with additional consideration paid to the complications of performing CRISPR/Cas9 editing in complex, often polyploid crop genomes, such as the need to target multiple paralogs and the need for unique validation primers.

Results

- The code for CROPSR was written in Python 3.7 and is available on Github. One of the main advantages of CROPSR is that it was developed as a single, modular, self-contained tool that performs all steps in the experimental design — from identifying the PAM sites located in the genome, to the design of PCR primers for experimental validation.
- Our gRNA scoring model provides a significant increase in prediction accuracy over existing tools, even in non-crop genomes.



Overview of a CRISPR experiment using CROPSR Timeline and steps of a typical CRISPR/Cas9 knockout experiment in a crop plant genome, utilizing CROPSR. Steps contained in gray blocks represent steps that only need to be done once per genome, at the first utilization of CROPSR (database generation). Consecutive uses on the same genome require only a database search, as shown.

Significance

- The release of CROPSR is the first step on a longer path to provide an innovative, open-source toolkit to assist in the design of CRISPR experiments and other manipulations of crop genomes.
- CROPSR provides the scientific community with new methods and a new workflow for performing CRISPR/Cas9 knockout experiments. CROPSR reduces the challenges of working in crops and helps speed gRNA sequence design, evaluation and validation. The new software may accelerate discovery and reduce the number of failed experiments.

Müller Paul, H., et al. Feb. 16, 2022. "CROPSR: An Automated Platform for Complex Genome-Wide CRISPR gRNA Design and Validation." BMC Bioinformatics 23: 74. DOI: 10.1186/s12859-022-04593-2.

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