Abstract

Genome-wide loss-of-function genetic screens are a powerful way to identify novel protein function and biological processes within a cell. A common approach in in-vitro loss-of-function screening to knock out genes in a population of cells, apply a stressor, and screen for surviving cell populations is to use a lentiviral vector to deliver a CRISPR/Cas9 system. The system has been modified to maximize the selectivity of the selected population relative to a control. The easy programmability and high throughput efficiency of the CRISPR/Cas9 system has made it easier to maximize the potential of this in-vitro screening method to identify genes responsible for a given phenotype of interest. Current methods using pooled sgRNA libraries in loss-of-function screens rely on lentiviral vector delivered delivery followed by next-generation sequencing (NGS) to analyze the resulting distribution of sgRNA sequences in screened cell populations. However, challenges include maintaining sgRNA representation in lentiviral production, achieving optimal titers upon scale-up of lentiviral production, and preparing high-quality NGS libraries that accurately reflect the distribution of sgRNA sequences.

Here we present a streamlined approach for producing Cas9-sgRNA cell populations in sufficient quantities for a genome-wide screen, and for generating NGS libraries used to analyze changes in sgRNA representation, using the Guide-It CRISPR Genome-Wide sgRNA Library System. Our methods enable even novice users to perform genome-wide phenotypic screens without concerns for sgRNA representation, low virus titer, or NGS library preparation.

Methods

Generation of sgRNA library and Cas9-expressing lentivirus: XCI™ pool vector containing target sequences and Cas9 for expression through a lentiviral vector were combined into a transfection mix and lyophilized in a single step, using the Guide-It CRISPR Genome-Wide sgRNA Library System. Our methods enable even novice users to perform genome-wide phenotypic screens without concerns for sgRNA representation, low virus titer, or NGS library preparation.

Validation of sgRNA activity: Following the generation of a Cas9-sgRNA lentiviral vector population using the method described above, random clones were selected, screened for corresponding sgRNA sequences, and cleavage of genomic targets assessed by resolvase assay. sgRNA activity was further demonstrated by performing a screen for resistance to the purine analog 6-thioguanine (6-TG). Finally, a comprehensive workflow for gDNA purification and sgRNA sequence amplification (the Guide-It CRISPR Genome-Wide NGS Analysis Kit) was developed to minimize issues associated with PCR primer design, gDNA isolation, and amplification of sgRNA sequences.

Conclusion

CRISPR/Cas9 knockout screens are a powerful way to identify genes involved in a phenotype of interest but require transcription with a representative lentiviral sgRNA library. Many find that the production of a representative sgRNA library is a major and labor-intensive step that can be very challenging. Simply with the addition of water, the Guide-It CRISPR Genome-Wide sgRNA Library System provides a simplified process for obtaining a representative, high-titer lentiviral sgRNA library with a high titering efficiency. NGS libraries were easily produced from 6-TG screen populations using the Guide-It CRISPR Genome-Wide NGS Analysis Kit.

Editing activity of the Guide-It CRISPR Genome-Wide sgRNA Library System was assessed in randomly selected clones as well as a 6-thioguanine screen.