Reviewer Report

Title: GuideMaker: Software to design CRISPR-Cas guide RNA pools in non-model genomes

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Reviewer Comments to Author:

Summary:
In this manuscript, Poudel et al. present a software, GuideMaker, to rapidly design sgRNAs targeting non-model genomes. Various input parameters such as PAM motif, guide length, length of seed region for off-target searching and so on can be toggled to design a panel of sgRNAs for pooled screening projects. The tool also helps pick control sgRNAs to include in the sgRNA pool. To benchmark the computational performance of their tool, the authors used GuideMaker to design sgRNAs targeting E.coli, P.aeruginosa, Aspergillus fumigatus and Arabidopdis thaliana. They also compared GuideMaker to the existing design tool, CHOPCHOP and reported that the targets identified by GuideMaker were mostly similar to those identified by CHOPCHOP. This tool can be used as a stand-alone web application, command-line software or in the CyVerse Discovery Environment.
Overall, the tool is very well documented and easy to use. In the current version of the manuscript, GuideMaker does not show a clear improvement over the state-of-the-art design tool, CHOPCHOP. The authors do not implement any existing on-target scoring methods to determine the targeting efficacy of the picked sgRNAs. This can lead to picking guides that are highly specific but not effective enough.
Major points:
1. Implementing on-target scoring methods, at least for the Cas enzymes that have on-target efficacy information, can help improve the process of picking sgRNAs. This tool will probably be used more often with standard Cas enzymes and it will be useful to have on-target efficacy scores attached to the guide RNAs.
2. The authors do a thorough analysis of the computational performance of GuideMaker with various genomes and Cas enzymes but including a comparison of the computational performance of GuideMaker vs. CHOPCHOP will strengthen the manuscript.
3. The authors define the PAM sequence of SaCas9 to be NGRRT whereas the canonical PAM sequence of SaCas9 is NNGRRT. This should be modified throughout the manuscript and analyses involving SaCas9 should be redone.
4. A good addition to the tool would be to output a file with all the sequences that were designed targeting the region of interest with the specific PAM sequence. This gives the user a sense of the universe from which the final guides were picked.
5. Another useful input parameter would be to specify a target region that the user wants to focus on such as letting the user input genomic coordinates or a gene name or locus tag. For example, CRISPy by Blin et al., 2016 takes a GenBank file as input and allows the user to input features specific to the uploaded genome.
Minor points:

1. "CyVerse" is misspelled as "CyCVerse" in multiple places in the manuscript.
2. Reference Figure 2 in Line 92.
3. Line 154: "Ratios between tools were calculated by dividing the number of gRNA identified.."
4. In Supplementary Figure 3 "wit haVX2" should be "with aVX2".
5. GitHub link in Line 336 does not work.
6. Line 225-226: "GuideMaker also creates off-target gRNAs for use as negative controls in high-throughput experiments." "Off-target gRNAs" is misleading in this context.

Level of Interest

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