**Mycobacterial CRISPRi Primer Designer**

I. Description

This is a program designed to facilitate the creation of primers for CRISPR interference in *Mycobacteria*. It uses the primer design guidelines set forth in Rock et al. *Nat Micr* (2017) to build forward and reverse primers that anneal each other and have overhangs suited for cloning into BsmBI-linearized pJR962. In brief, the algorithm works as follows:

- search the user-input coding sequence for each of the strong PAM sites outlined in the paper

- for each strong PAM site found, if it is far enough from the end of the gene to actually design primers against, it will design the primers

- if no strong PAM sites are found, it gives the user the option to look for weaker PAM sites and design primers for those

- if no weak PAM sites are found, it reports that the input sequence cannot be knocked down using this exact technique

In addition to designing CRISPRi primers, the program can design primers for CRISPR. In practice, for the user, this is as simple as selecting a check button in the UI. For the program, this adds additional steps, such as:

- reverse complement the input coding sequence to create a template sequence

- if no strong PAM sites are found in either coding or template sequences, the user may again opt to search for weaker PAM sites (this time in both sequences)

- if no weak PAM sites are found, it reports that the input sequence cannot be targeted by CRISPR on either strand

As this program is merely a tool designed to speed up the process of designing CRISPR(i) sgRNA primers (primarily by finding the PAM sites for you), you should always check the primers returned against your sequence (i.e. using CLC Genomics Workbench) to verify that they make sense and are likely to achieve your desired knockdown/knockout.

Any bugs encountered should be reported as soon as possible. This can be done either by creating an issue on Github (<https://www.github.com/chg60/crispri/>) or by emailing Christian directly at [chg60@pitt.edu](mailto:chg60@pitt.edu) with “CRISPRi Primer Finder – Bug” in the subject line. Regardless of reporting method, please be sure to include a description of the application behavior, the name you gave your sequence, the coding sequence you entered, the program version number, and indicate whether the CRISPR box was checked and whether a save file was selected.

Please feel free to also send along suggestions for added functionality.

II. Guided Example

Upon double-clicking the application, a window should launch closely resembling the following:

The top entry box is there for you to name your sequence. It is strongly recommended that you give your sequence a name if you plan to save any primers to a file. My personal preference is to use whatever the gene name is. For example, if I’m knocking down gene 30 from Mycobacterium phage MichelleMyBell, I would name it MMB30 in the program.

The middle entry box is there for you to paste or type the *coding* sequence of your gene/region of interest. This is important because the CRISPRi system described in Rock et al. *Nat Micr* (2017) is strand-specific.

The “Choose save file” button is present for users that like to keep everything neatly documented and don’t want to copy and paste everything themselves. While users are not required to choose a save file, the program will prompt you to confirm that you want to proceed, should you choose to “Run Program” without choosing a save file.