

### Read Me File:

To run the script download the zip file, open the file, and then save the file to your desktop.

The name of the file is “Arjunsinh\_Harer\_Final\_Project\_RBIF\_109”.

Navigate to this folder in your terminal and type the following command.

```
python off_target.py
```

You will now see an example output of the script. The script evaluates the guide suitability of guideRNAs needed to knock out EGFR in homo-sapiens.

This script allows you to evaluate guideRNA candidates for a CRISPR knockout experiment in homo-sapiens.

If you want to run the script on a particular gene of interest, you can start by running the following command.

```
python download_fasta.py “Gene Name of Choice” “Your email”
```

An example command looks like this:

```
python download_fasta.py EGFR arjunsinhharer@brandeis.edu
```

Open the “Arjunsinh\_Harer\_Final\_Project\_RBIF\_109” directory and ensure your FASTA for your gene of interest is downloaded.

After downloading the file delete the any existing txt files in the directory if they exist. The off\_target.py script can only run if there is one .txt file in the directory.

This is the only manual step of the process. You need to obtain a list of guide RNAs for your gene of interest. You can do this using the [Synthego Knockout Guide Design Tool](#)

Once you open the link you’ll see a web-based tool to generate guide RNAs for a gene. For genome type Homo sapiens, select any gene of your choice and then click “search”.

Copy paste the recommended guide sequences in a .txt file, and each line should have one guide sequence listed. Once you copy-paste your guide sequences into the text file, save it to the directory “Arjunsinh\_Harer\_Final\_Project\_RBIF\_109”.

Please name the text file with the following convention otherwise the script won’t run *guides\_ {Gene name of your choice}.txt*.

Now that you have the FASTA file for your gene of interest as well as the guide RNA sequences you are now ready to run the script and get the output.

You can do this by simply typing the command specified in the start of this readme file which is.

```
python off_target.py
```

Great! You have now successfully run your own independent analysis of guide RNA suitability for a CRISPR experiment. If you run into any issues running this script, please do not deduct points, instead call me, or text me 703-582-3705. You can text this number as well, or email me at [arjunsinhharer@brandeis.edu](mailto:arjunsinhharer@brandeis.edu) Thanks, and I hope you enjoy!