**High throughput CRISPR design**

A step-by-step guide for generating sgRNAs for injecting into C. elegans from a list of protein IDs, WormBaseIDs or gene names.

Adapted from (Gagnon et al., 2014)

Required software and APIs (and how to install included in guide):

Intermine

ChopChop

Primer3

1. It is advisable to run Intermine and Chopchop within a virtual environment in order to avoid conflicts with operating system python. In terminal:
2. conda create -n chopchop python=2.7
3. source activate chopchop
4. conda install pandas numpy scipy scikit-learn==0.16.1
5. pip install biopython
6. pip install intermine
7. Now clone the chopchop repository into a directory in your home folder (or wherever you normally save downloaded repos):

In terminal:

1. mkdir chopchop
2. cd \*/chopchop
3. git clone https://bitbucket.org/valenlab/chopchop.git
4. rm -rf bowtie
5. rm -r twoBitToFa primer3\_core
6. Download the .2bit genome file (either directly from UCSC or using wget, which can be installed via HomeBrew)
   1. http://hgdownload.cse.ucsc.edu/downloads.html
7. cd \*/chopchop
8. wget http://hgdownload.cse.ucsc.edu/gbdb/ce11/ce11.2bit
9. Download twoBitoFa and convert 2bit genome file to .fa files.
10. wget http://hgdownload.soe.ucsc.edu/admin/exe/macOSX.x86\_64/twoBitToFa
11. chmod 744 twoBittoFa
12. ./twoBittoFa ce11.2bit ce11.fa
13. mkdir 2bitFolder
14. mv -f \*.2bit 2bitFolder/
15. Download bowtie (either from sourceforge or via wget) and convert ce11.fa to ewbt files:

In terminal

1. cd \*/chopchop
2. wget https://sourceforge.net/projects/bowtie-bio/files/bowtie/1.0.1/bowtie-1.0.1-macos-x86\_64.zip
3. unzip bowtie-1.0.1-macos-x86\_64.zip
4. mv bowtie-1.0.1 bowtie
5. cd \*/bowtie
6. chmod + bowtie
7. chmod + bowtie-build
8. cp -f bowtie-build ..
9. cd ..
10. ./bowtie-build ce11.fa ce11
11. cd ..
12. mkdir ebwtFiles
13. mv -f \*.ebwt /ebwtFiles
14. download primer3
15. git clone https://github.com/primer3-org/primer3.git primer3
16. cd primer3/src
17. make
18. make test
19. chmod 744 primer3\_core
20. cp -f primer3\_core ..
21. Download gene table from UCSC (<http://genome.ucsc.edu/cgi-bin/hgTables?command=start>)

* Select organism and assembly
* Select group: Genes and Gene Predictions
* Select track: RefSeq Genes or Ensemble Genes
* Select table: refFlat or ensGene
* Select region: genome
* Select output format: all fields from selected table
* Fill name with extension ".gene\_table' eg. danRer10.gene\_table
* Get output

1. Move to folder called ‘GeneTables’
2. cd \*/chopchop
3. mkdir GeneTables
4. mv -f \*.gene\_table /GeneTables
5. Open chopchopy.py and set the pathnames for:
6. .2bitgenomefiles (eg. TWOBIT\_INDEX\_DIR = "/Users/ibarlow/repositories/chopchop/2bitFolder")
7. .ewbt files (eg. BOWTIE\_INDEX\_DIR = "/Users/ibarlow/repositories/chopchop/ebwtFiles")
8. .gene\_tables (eg. GENE\_TABLE\_INDEX\_DIR = "/Users/ibarlow/repositories/chopchop/GenePred")

Preparing chopchop input

1. Pull repo https://github.com/ilbarlow/DiseaseModelling.git
2. git clone https://github.com/ilbarlow/DiseaseModelling.git
3. cp -f WormMineSequenceExport.py ..
4. Run the WormMineSequenceExport.py to generate gDNA, cDNA sequences, sgRNA input file and FASTA.txt file (optional)
   1. The output sgRNAInput.csv should be sufficient for use in fast Chopchop.py
5. python wormmine\_sequence\_export.py /path/to/input/WormIDs [margin aroundStart] /output/directory/forSequences createFasta=Boolean
6. Run fast\_chopchop.py to generate putative crRNA targets

In terminal:

1. cd \*/chopchop
2. python fast\_chopchop.py ‘PathName/to/your/sgInput.csv’ optional extra inputs (eg -P. -f GG -3 PRODUCT\_SIZE\_MAX=200

/// see ./chopchop.py –help for more information)

A folder in the same directory as your sgInput.csv will be created containing all the results

1. Create a list of the sgRNA sites as a txt file to be imported into ape for annotation
2. python chopchop\_to\_features.py /path/to/your/Chopchopoutput/directory /path/to/savedirectory/for/features\_files.txt typeof\_sgRNA (GA/GG/General)
3. Open individual gDNA and cDNA sequences and import the feature lists

TODO: Once the guide sites have been selected