
CLASHChimeras Documentation

Release 0.1b3

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Mar 21, 2017

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CLASHChimeras is a [Python](#) package for analysing [CLASH](#) datasets. It takes raw fastq files as input and provides comprehensive analysis of RNA profiles and chimeric reads identification. The output is [CSV](#) and [BED](#) format files for easy visualization in Genome Browsers.

CHAPTER 1

Installation

You can install it using [pip](#) after you have setup Python version 3.4 or above. Please use this [guide](#) for setting up [Python](#) if you have not done it already. After setting up [Python](#) and [pip](#), you can run this on your shell

For local installation (Usually `$HOME/.local`):

```
$ pip3 install --user CLASHChimeras
```

For global installation (Usually `/usr/`):

Note: You should have `sudo` privileges

```
$ sudo pip3 install CLASHChimeras
```


CHAPTER 2

Dependencies

Warning: These dependencies must be satisfied if you want to use *align-for-chimeras*

CLASHChimeras requires certain software to be installed and setup before you can use it completely. The software you need to explicitly install are the following:

- [Bowtie2](#) - Fast and sensitive read alignment
- [Tophat](#) - A spliced read mapper for RNA-Seq

The package can be used by three executable scripts:

1. *download-for-chimeras*
2. *align-for-chimeras*
3. *find-chimeras*

download-for-chimeras

align-for-chimeras

Warning: The input fastq is expected to be adapter trimmed and quality controlled

Note: *Flexbar* can be used to trim raw fastq sequences

find-chimeras

Note: It's recommended that you provide SAM files as input which are generated using *align-for-chimeras*

Todo

Provide support for detecting chimeras between same RNA types

Example

We will be using the a dataset from [CLASH](#) experiment which is hosted [here](#)

In this instance, we'll be using the first 4 million reads from the dataset. The sequential order to find chimeras on [CLASH](#) datasets using this package is the following:

Run `download-for-chimeras`

Run `download-for-chimeras` for the first time to download sequences and generate necessary indexes

The dataset that we are using here belong to *H. sapiens*. The sequence database needs to be downloaded from [Gencode](#) and [miRBase](#). Here's how you can download:

The code below assumes the default path as `~/db/CLASHChimeras` but if you want a different folder to put your sequences, please specify it using `--path /path/to/your/folder` as a argument. It's highly recommended to get yourself familiar with the arguments by typing `download-for-chimeras -h`

```
$ download-for-chimeras -gor "H.sapiens" -mor hsa
```

Note: It's an interactive script which prompts for user input when selecting the release version.

Warning: Please be patient as this is a big download and index generation takes even longer

Warning: The latest release from Gencode when downloaded and after all indexes are generated, takes around 11G of space

Below is an example of how `download-for-chimeras` runs.

Note: All the database files are already present in this example run, so they are verified by sha256sums. Thus, the timestamps are very close to each other. Actual download and generation of indexes will take a while

Indexes

There are a series of [bowtie2](#) and [tophat](#) indexes generated after you've run `download-for-chimeras` script. Assuming that you ran the command below and selected the latest versions of [Gencode](#) and [miRBase](#), the following indexes will be generated automatically

```
$ download-for-chimeras -gor "H.sapiens" -mor hsa
```

smallRNA & targetRNA Indexes

These indexes can be used as `--smallRNAIndex -si` or `--targetRNAIndex -ti` in *align-for-chimeras*

Path for index	Index Type	RNA Type
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.pc_transcripts	Bowtie2	protein_coding
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.lncRNA_transcripts	Bowtie2	lncRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.snoRNA_transcripts	Bowtie2	snoRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.snRNA_transcripts	Bowtie2	snRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.tRNA_transcripts	Bowtie2	tRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.misc_RNA_transcripts	Bowtie2	misc_RNA
~/db/CLASHChimeras/Mirbase/21/hsa-hairpin	Bowtie2	miRNA-hairpin
~/db/CLASHChimeras/Mirbase/21/hsa-mature	Bowtie2	miRNA-mature

Genome-Index

This index should be provided if you run `align-for-chimeras` with `--run tophat`

Path for index	Type
~/db/CLASHChimeras/Gencode/H.sapiens/22/GRCh38.p2.genome	Bowtie2

Transcriptome-Index

This index should be provided if you run `align-for-chimeras` with `--run tophat` along with *Genome-Index*

Path for index	Type
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation	tophat

Annotation

Annotation File	RNA type
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf	protein_coding
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf	lncRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf	snRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf	snoRNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf	misc_RNA
~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.tRNAs.gtf	tRNA
~/db/CLASHChimeras/Mirbase/21/hsa.gff3	miRNA

Run align-for-chimeras

Note: Please refer to [Indexes](#) when selecting `--smallRNAIndex -si` or `targetRNAIndex -ti` when you run *align-for-chimeras*

For this instance, we want to find the chimeras between miRNA and protein_coding from the raw reads. After you have successfully run *download-for-chimeras* and made sure that all the indexes are present for your alignment to begin, please use the following command

```
$ align-for-chimeras -i E3_4M.fastq.gz -gz -r bowtie2 -si ~/db/CLASHChimeras/Mirbase/
↳ 21/hsa-hairpin -ti ~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.v22.pc_
↳ transcripts -o E3-miRNA-pc
```

This is how it runs.

After the successful execution of *align-for-chimeras*, these are the files that are generated

- E3-miRNA-pc.smallRNA.sam
- E3-miRNA-pc.targetRNA.sam

Note: Please use `--threads -n` to specify the number of cores to use when executing *Bowtie2*

align-for-chimeras also provides an argument to run *tophat* as well. This helps in visualise the transcript coverage across the genome. Please use the following command to align to the whole genome

```
$ align-for-chimeras -i E3_4M.fastq.gz -gz -r tophat -gi ~/db/CLASHChimeras/Gencode/H.
↳ sapiens/22/GRCh38.p2.genome -tri ~/db/CLASHChimeras/Gencode/H.sapiens/22/gencode.
↳ v22.chr_patch_hapl_scaff.annotation -o E3_4M
```

To create *bigWig* file from the *tophat* output, I'd recommend using *deepTools* to create normalized coverage file from the following [wiki page](#)

Let's move forward with finding chimeras between these RNA types

Run find-chimeras

Note: Please refer to [Annotation](#) when selection `--smallRNAAnnotation -si` or `--targetRNAIndex -ti` when you run *find-chimeras*

Following up after running `align-for-chimeras`, it's time to detect chimeras. Please make sure that you have the SAM files generated from `align-for-chimeras`, please use the following command

```
$ find-chimeras -s E3-miRNA-pc.smallRNA.sam -t E3-miRNA-pc.targetRNA.sam -ggs -sa ~/
↪db/CLASHChimeras/Mirbase/21/hsa.gff3 -ggt -ta ~/db/CLASHChimeras/Gencode/H.sapiens/
↪22/gencode.v22.chr_patch_hapl_scaff.annotation.gtf -o E3-miRNA-pc
```

This is how the above command runs

After the successful execution of `find-chimeras`, these are the files that are generated

- E3-miRNA-pc.chimeras.tsv
- E3-miRNA-pc.smallRNA.bed
- E3-miRNA-pc.targetRNA.bed

Note: Please note if you have not specified `--getGenomicLocationsSmallRNA -ggs, <sample>.smallRNA.bed` will not be generated. If you haven't specified `--getGenomicLocationsTargetRNA -ggt, <sample>.targetRNA.bed` will not be generated.

You can view the chimeras from the `<sample>.chimeras.tsv` file that is generated. If you want to visualize the data in genome browsers, you can do that by adding the `<sample>.smallRNA.bed` and `<sample>.targetRNA.bed` in the [IGV](#) or your genome browser of choice.

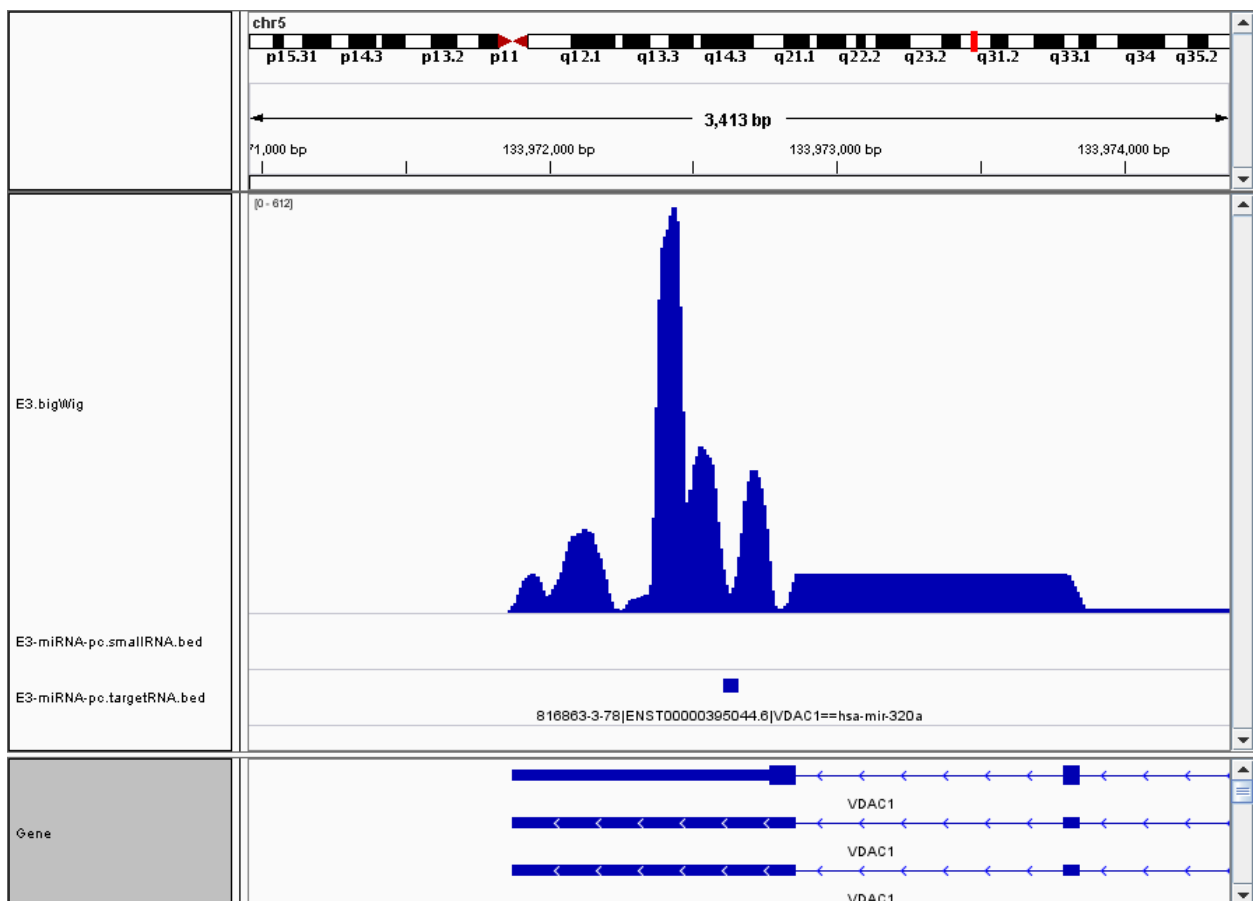
Note: Please check the genome assembly version described in [Genome-Index](#) and make sure you have the same or corresponding version set in your genome browser

Possible combinations

Because of the modular design of the software, it is possible to find chimeras between different types of RNA. Please refer to [Indexes](#) and run *align-for-chimeras* with the smallRNA and targetRNA of your choice.

Visualisation in Genome Browser

This is an example visualization in IGV with the normalized coverage included as a track



CHAPTER 6

Chimeras table

Here is the example chimeras table that is generated. The columns information can be found **commented** in the first lines

CHAPTER 7

Issues & Feedback

If you encounter any issues, please report it on the [Issues](#) page of the Github [repository](#). Please feel free to offer your suggestions and feedback and contribute by submitting pull requests.