CLASHChimeras Documentation

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Kashyap Chhatbar

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Contents

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

CHAPTER 3

Usage

The package can be used by three executable scripts:

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

3.1 download-for-chimeras

Downloads required sequences and create bowtie2 indexes required for alignment

```
usage: An example usage is: download-for-chimeras -gor ``H.sapiens'' -mor hsa
```

Options:

--gencodeOrganism=H.sapiens, -gor=H.sapiens Select model organism

Possible choices: H.sapiens, M.musculus

--mirbaseOrganism=hsa, -mor=hsa Select organism to download microRNAs for

--path=~/db/CLASHChimeras, -pa=~/db/CLASHChimeras Location where all the database files and indexes will be downloaded

--logLevel=INFO, -l=INFO Set logging level

Possible choices: INFO, DEBUG, WARNING, ERROR

--bowtieExecutable, -be Provide bowtie2 executable if it's not present in your path

--tophatExecutable, -te Provide Tophat executable if it's not present in your path

--miRNA=hairpin, -mi=hairpin Which miRNA sequences to align

Possible choices: mature, hairpin

3.2 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

Given a fastq file, this script executes bowtie2 and tophat aligners to generate alignment files necessary for detecting chimeras in the reads

```
usage:
 align-for-chimeras -i input.fastq -si /path/to/smallRNA_index -ti /path/to/targetRNA_index
 align-for-chimeras -i input.fastq -gi /path/to/genome_index -tri /path/to/transcriptome_in


 To see detailed help, please run
 align-for-chimeras -h
```

Options:

--input, -i Input file containing reads fastq

--smallRNAIndex, -si Provide the smallRNA bowtie2 index (Usually resides in ~/db/CLASHChimeras or elsewhere if you have specified in –path -pa during initialize)

--targetRNAIndex, -ti Provide the targetRNA bowtie2 index (Usually resides in ~/db/CLASHChimeras or elsewhere if you have specified in –path -pa during initialize)

--genomeIndex, -gi Provide the genome bowtie2 index (Usually resides in ~/db/CLASHChimeras or elsewhere if you have specified in –path during initialize)

--transcriptomeIndex, -tri Provide the transcriptome index as specified in tophat –transcriptome-index

--output, -o The output name without extension (.sam .bam will be added)

--run=bowtie2, -r=bowtie2 Run the following aligner for raw reads

Possible choices: bowtie2, tophat

--logLevel=INFO, -l=INFO Set logging level

Possible choices: INFO, DEBUG, WARNING, ERROR

--gzip=False, -gz=False Whether your input file is gzipped

--bowtieExecutable, -be Provide bowtie2 executable if it's not present in your path

--tophatExecutable, -te Provide Tophat executable if it's not present in your path

--preset=sensitive-local, -p=sensitive-local Provide preset for bowtie2

Possible choices: very-fast, fast, sensitive, very-sensitive, very-fast-local, fast-local, sensitive-local, very-sensitive-local

--tophatPreset=very-sensitive, -tp=very-sensitive Provide preset for Tophat

Possible choices: very-fast, fast, sensitive, very-sensitive

--mismatch=1, -m=1 Number of seed mismatches as represented in bowtie2 as -N

Possible choices: 0, 1

--reverseComplement=False, -rc=False Align to reverse complement of reference as represented in bowtie2 as –norc

--unaligned=False, -un=False Whether to keep unaligned reads in the output sam file. Represented in bowtie2 as –no-unal

--threads=1, -n=1 Specify the number of threads

3.3 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

Given two SAM files, this script tries to find chimeras that are observed between a smallRNA and a targetRNA

```
usage: An example usage is: find-chimeras -s smallRNA.sam -t targetRNA.sam -o output
```

Options:

--smallRNA, -s Provide smallRNA alignment SAM file

--targetRNA, -t Provide targetRNA alignment SAM file

--getGenomicLocationsSmallRNA=False, -ggs=False Do you want genomic locations for small RNA?

--getGenomicLocationsTargetRNA=False, -ggt=False Do you want genomic locations for target RNA?

--smallRNAAnnotation, -sa Provide smallRNA annotation gtf(from Gencode) or gff3(from Mirbase). Only provide gtf from Gencode or gff3 from Mirbase. Does not support other gtf files

--targetRNAAnnotation, -ta Provide targetRNA annotation gtf(from Gencode). Only provide gtf from Gencode. Does not support other gtf files

--output, -o The output name without extension (.bed .csv will be added)

--overlap=4, -ov=4 Maximum overlap to be set between two molecules when determining chimeras

--gap=9, -ga=9 Maximum gap (number of unaligned nucleotides) to allowed between two molecules within a chimera

--logLevel=INFO, -l=INFO Set logging level

Possible choices: INFO, DEBUG, WARNING, ERROR

CHAPTER 4

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:

4.1 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

4.2 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
```

4.2.1 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

4.2.2 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

4.2.3 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

4.3 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

4.4 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 -
```

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/GRCh
```

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

4.5 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/
```

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.

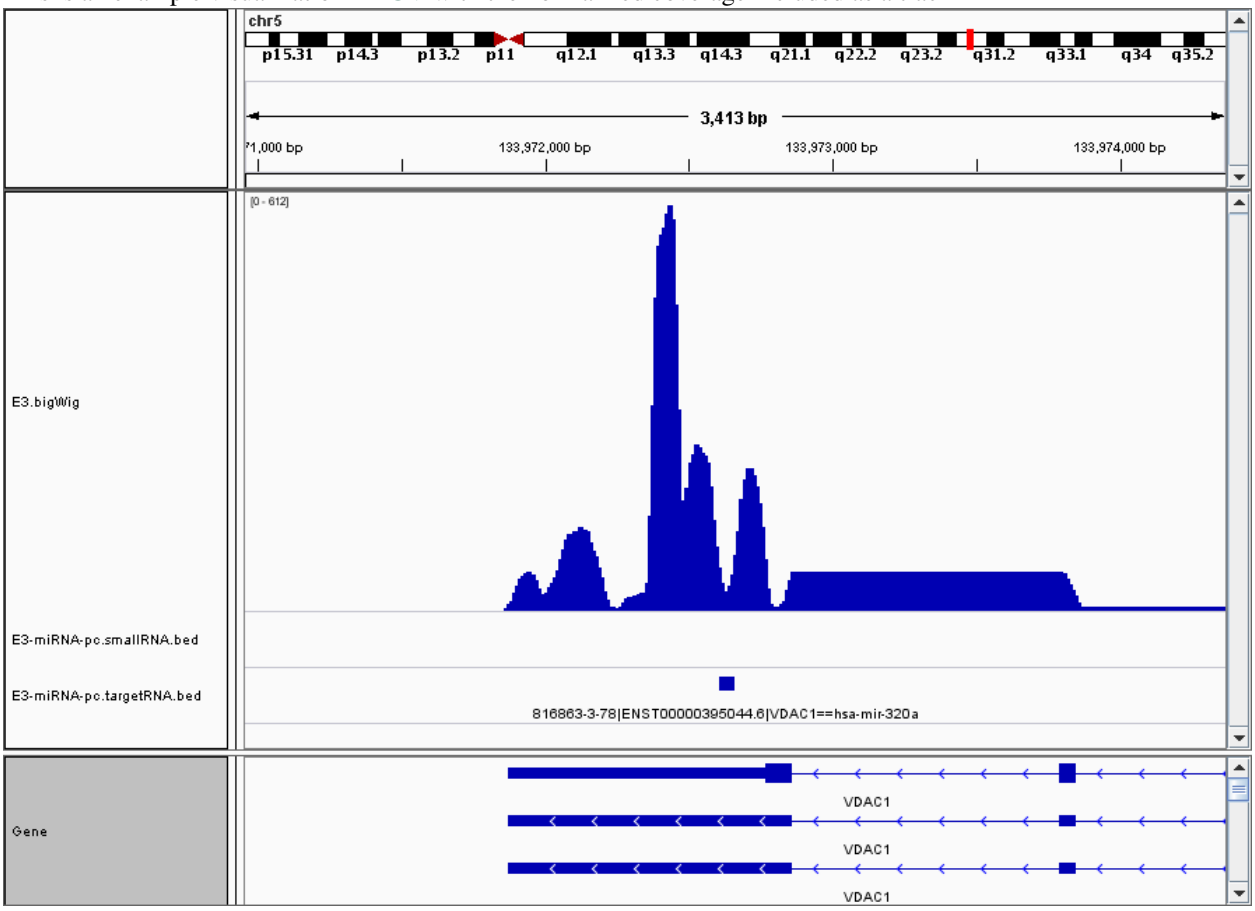
Note: Please check the genome assembly version described in *Genome-Index* and make sure you have the same or corresponding version set in your genome browser

4.6 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.