

iCLIPit

Version

1.0

Authors

Anastassiya Zidkova and Martin Zidek

Description

Code in python and test data for RNA iCLIP analysis. this repository contained 4 scripts:

- iclipBarplot.py
- iclipBoxplot.py
- iclipFdr.py
- iclipRealFreq.py
- iclipZscores.py

Example data and results are in ToyExample folder.

Installation

Clone git repository by following command `< git clone https://github.com/anastazie/iCLIP >`

Getting help

In order to get full list of parameters for function type: `< python <function_name> -h >`

Data preprocessing

SAM files

This package use only positions in sam file. Before running scripts extract first 4 columns without header using following command: `< grep -v "^@" test.sam |cut -f1,2,3,4 > test1_sub.sam >`

GTF files

Preprocess GFT file by removing header using following command: `< grep -v "^##" test.gtf > test_nohead.gtf >`

Get real frequencies

Description

Compute number of mapped reads starting at each genome position

Parameters

-f File containing first 4 columns of .sam files without header

Output

CSV file *realfreq.csv* containing two columns:

Command

```
< python iclipRealFreq.py -f test1_sub.sam >
```

Compute FDR (False Discovery Rate) threshold

Description

Compute FDR threshold by computing following rate: number of positions with n reads / mean number of positions with n reads across i iterations of randomized datasets

Parameters

-f File containing first 4 columns of .sam files without header

-g Corresponding annotation file in GTF format

-r Range of nucleotides in one direction from the position to calculate number of reads starting in the range: position \pm range_number, default value: 15

-i Number of iterations to generate randomized datasets from input file, default value: 100

Output

CSV file containing two columns:

Command

```
< python iclipFdr.py -f test1_sub.sam -g test_nohead.gtf -r 20 -i 10 >
```

Compute z-scores for k-mers

Description

Compute k-mer z-scores by its occurrence in randomized datasets.

Parameters

- f File containing first containing two columns:
- fa Fasta file containing reference nucleotide sequence in fa.tab format with two columns:
- g Corresponding annotation file in GTF format
- t FDR threshold value
- r Range of nucleotides in one direction from the position to calculate number of reads starting in the range: position +- range_number, default value: 15
- i Number of iterations to generate randomized datasets from input file, default value: 100
- k k-mer type: pentamer, hexamer, etc.
- l Length of sequence to output into seq.txt file: position +- length, default value: 10

Output

Two files are produced:

__seq.txt contains sequence for positions above threshold +- *l*

__zscores.csv contains three columns:

Command

```
< iclipZscores.py -f test1_sub.sam -fa test.fa.tab -g test_nohead.gtf -t 100 -r 15 -i 10  
-k 4 -l 5>
```

Create barplot with read fraction per each gene

Description

Create barplot by computing reads fraction started at each gene

Parameters

- f File containing first containing two columns:
- g Corresponding annotation file in GTF format
- n number of genes, default value: all genes detected in GTF file
- t plot title, default value: "Barplot"

Output

PNG file with barplot, x - gene name, y - reads fraction, %

Command

```
< iclipBarplot.py -f test1_sub_real_freq.csv -g test_nohead.gtf -n 5 -t testBar >
```

Get boxplot with reads fraction per each gene interval

Description

Create boxplot by computing dividing each gene into p parts and counting number of reads starting in each interval

Parameters

- f File containing first containing two columns:
- g Corresponding annotation file in GTF format
- n number of genes, default value: all genes detected in GTF file
- t plot title, default value: "Boxplot"
- p number of parts, into which all genes will be divide, default value: 100

Output

PNG file containing boxplot per each gene (distribution of reads number in each interval)

Command

```
< iclipBoxplot.py -f test1_sub_real_freq.csv -g test_nohead.gtf -n 5 -t testBox -p 100 >
```

References

1. Wagon, Jacy L., et al. "CELF4 regulates translation and local abundance of a vast set of mRNAs, including genes associated with regulation of synaptic function." PLoS genetics 8.11 (2012): e1003067.
2. König, Julian, et al. "iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution." Nature structural & molecular biology 17.7 (2010): 909-915.
3. Wang, Zhen, et al. "iCLIP predicts the dual splicing effects of TIA-RNA interactions." PLoS biology 8.10 (2010): e1000530.