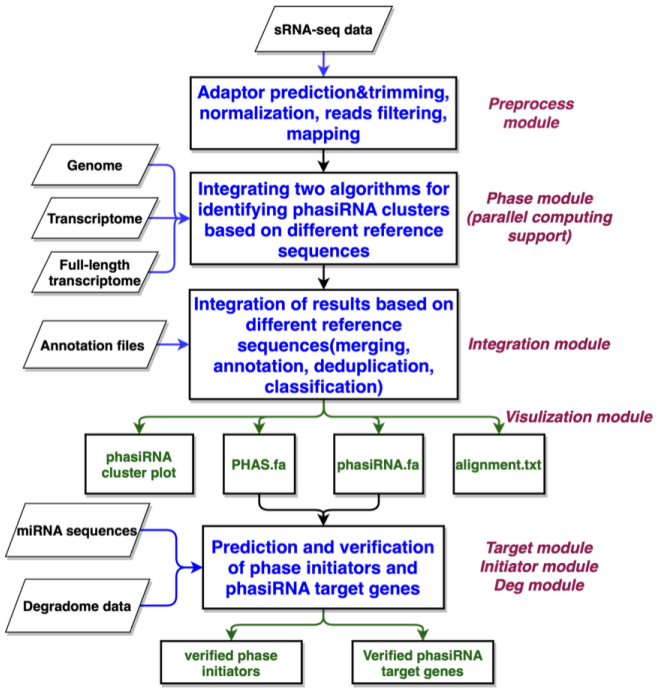
Index

User guide

Welcome to phasihunter 😉

A multithreaded program for mining phasiRNA regulation pathways based on multiple reference sequences.

PhasiHunter workflow



Dependencies

phasihunter is a CLI program runing on linux platform. The correction runing of phasihunter depends on some existing softwares.

- Bowtie (Langmead, et al., 2009. Genome Biol)
- Biopython (Cock, et al., 2009. Bioinformatics)
- Bedtools (Quinlan and Hall, 2010. Bioinformatics)
- Dnapi (Tsuji and Weng, 2016. PloS One)
- Trim_galore (https://github.com/FelixKrueger/TrimGalore)
- Seqkit (Shen, et al., 2016. PloS One)
- Perl5 (https://www.perl.org)
- Fasta36 (Pearson and Lipman, 1988. Proc Natl Acad Sci U S A)
- TarHunter (Ma, et al., 2018. Bioinformatics)

```
# Bowtie, Biopython, Bedtools, Trim_galore, Seqkit can install with conda conveniently.
conda insatll bowtie biopython bedtools trim-galore, seqkit -c conda-forge -c bioconda -n <your conda env name>

# Dnapi and TarHunter also include in PhasiHunter. Users do not need to download them separately

# Fasta36 provied binary package, user can download from github website
wget -c https://github.com/wrpearson/fasta36/releases/download/v36.3.8i_14-Nov-2020/fasta-36.3.8i-linux64.tar.gz
tar -xzvf fasta-36.3.8i-linux64.tar.gz
cd fasta-36.3.8i/bin
echo "export PATH=\$PATH:$PWD >> ~/.bashrc"

# perl5 is pre-installed software in most unix distributions
```

Installation

1. Clone phasihunter

   `git clone https://github.com/HuangLab-CBI/PhasiHunter.git .`
2. Setting enviroment variable in ~/.bashrc

   `echo "export PATH=\$PATH:<phasihunter PATH> >> ~/.bashrc"`

   example:

   `echo "export PATH=\$PATH:/home/user/volumes/PhasiHunter >> ~/.bashrc"`
3. type `phasihunter -h` to check phasihunter whether installation correct. If phasiHunter is installed correctly you will see the following content.

```
Usage:
    phasiHunter subcommand options

subcommand:
    run:            one command executing mode, config.yaml file required
    preprocess:     generating map file for phasiRNA cluster prediction
    phase:          predicting phasiRNA cluster based on multiple reference sequences
    integration:    integrating phase module output
    visulization:   for phasiRNA cluster visulization
    target:         for sRNA target gene prediction
    initiator:      for phasiRNA initiator prediction
    deg:            for phasiRNA initiator or phasiRNA target gene verrification based on degradome verrification

type phasiHunter subcommad -h for more subcommad detail
```

Executing PhasiHunter with step-by-step submodules.

Parameter in < > means necessary; parameter in [ ] means optional

1. Data pre-process

```
phasiHunter preprocess -m <r> -i <SRR5049781.fastq.gz> -r <oryza_sativa_cdna.fa> -o [SRR5049781_cdna.map]

phasiHunter preprocess -m <r> -i <SRR5049781.fastq.gz> -r <oryza_sativa_gdna.fa> -o [SRR5049781_gdna.map]
```

- preprocess module usage

```
Help messeage:
  options:
    # necessary options:
    -m:  string --  mode: r | c | m;
                    raw(mode): trim adaptor --> normalization --> length and abundance filter --> mapping
                    clean(mode): normalization --> length and abundance filter --> mapping
                    mapping(mode): mapping
    -i:  file   --  for r mode: fastq file or fastq.gz file
                    for c mode: fasta file or fasta.gz file
                    for m mode: length and abundance filter fasta file
    -r:  file   --  reference sequence fasta file
    -in: string --  index prefix, -r option will be ignored when -in enable
    -o:  outfile --  outfile name

    # options with default value
    -j:  int    --  adaptor trim parallel cores; <8 is recommend, only need in r mode, default=1
    -bj: int    --  bowtie parallel cores; defalut=1
    -mh: int    --  max hits when mapping to ref sequence, default=10
    -mi: int    --  minimal sRNA reads length cutoff, default=19
    -ma: int    --  maxmial sRNA reads length cutoff, default=25
    -e:  float  --  sRNA reads cpm cutoff, default=1
    -n:  int    --  normalization base, default=1000000

```

```
    # other
    -v:         --  print version information
    -h:         --  print help information
```

2. PhasiRNA and PHAS loci prediction

```
phasiHunter phase -cm <SRR5049781_cdna.map> -c <oryza_sativa_cdna.fa> -gm
<SRR5049781_gdna.map> -g <oryza_sativa_gdna.fa> -fa
<SRR7851621_trimmed_format_filter.fa> -a [SRR5049781_allsiRNA.txt] -o
[SRR5049781_phasiRNA.txt] -pl [21] -j [10] -pv [0.0001] -ps [15] -pr [0.4]
```

- phase module usage

```
phase usage:
  option:
    -cm: file  --  map file based on reference transcriptome sequence
    -c:  file  --  reference transcritome sequence, fasta file
    -gm: file  --  map file based on reference genome sequence
    -g:  file  --  reference genome sequence, fasta file
    -fm: file  --  map file based on full length transcriptome sequence
    -f:  file  --  full length transcriptome sequence, fasta file
    -fa: file  --  sRNA file
    -a:  out   --  allsiRNA cluster output file, default name is phase_a.txt
    -o:  out   --  phasiRNA cluster output file, default name is phase_o.txt
    -me: str   --  phasiRNA prediction method, h(hypergeometric test) |
p(phase score) | b (both), default=b
    -il: int   --  phasiRNA cluster island, default=5
    -pl: int   --  phase length, 21 | 24, default=21
    -pn: int   --  phase number, default=4
    -mh: int   --  max hits when mapping to ref sequence, default=10
    -j:  int   --  parallel number, default=1
    -pv: float --  pvalue cutoff, default=0.001, only function with h/b
method applied
    -ps: float --  phase score cutoff, default=15, only function with p/b
method applied
    -pr: float --  phase ratio cutoff, default=0.4, only function with p/b
method applied
    -cl: str   --  delete .phasiHuter_bowtieIndex, y|n, default=y
    -v:        --  print version information
    -h:        --  print help information
```

3. PhasiRNA and PHAS loci result integration

```
phasiHunter integration -io <SRR5049781_phasiRNA.txt> -ia
<SRR5049781_allsiRNA.txt> -an <oryza_sativa_gdna.gff3> -o
[SRR5049781_phasiRNA_dup.txt] -a [SRR5049781_allsiRNA_dup.txt] -s
[SRR5049781_summary.txt] -po [SRR5049781_phas.txt] -g <y>
```

- integration module usage

```
integration usage:
  option:
    # necessary options:
    -io: file  --  phase module -o output file
    -ia: file  --  phase module -a output file
    -an: file  --  reference genome gff3 file
    -g:  str  --  y | n, whether exist gdna based PHAS loci

    # options with default value
    -o:  out  --  integration phasiRNA cluster, default name is integration_o.txt
    -a:  out  --  integration all siRNA cluster, default name is integration_a.txt
    -s:  out  --  integration summary, default name is integration_s.txt
    -po: out  --  PHAS loci information, default name is integration_p.txt
    -j:  int   --  parallel number, default=1
    -pn: int   --  phase number, default=4
    -pl: int   --  phase length, 21 | 24, default=21
    -pv: float --  pvalue cutoff, default=0.001
    -il: int   --  phasiRNA cluster island, default=5
    -dp: str   -- y | n, discard only P method result, default=y

    # optional options
    -fn: file  --  full length transcript annotation file

    # other
    -v:       --  print version information
    -h:       --  print help information
```

4. Print phasiRNA_cluster plot, phasiRNA.fa, PHAS.fa

```
phasiHunter visulization -io <SRR5049781_phasiRNA_dup.txt> -ia <SRR5049781_allsiRNA_dup.txt> -ip <SRR5049781_phas.txt> -a [SRR5049781_alignment.txt] -o [SRR5049781.phasiRNA.fa] -p [SRR5049781.PHAS.fa] -c [oryza_sativa_cdna.fa] -g [oryza_sativa_gdna.fa] -pc [y] -pg [y]
```

- visulization module usage

```
visulization usage:
  option:
    # necessary options:
    -io: file  --  integration -io outputfile
    -ia: file  --  integration -ia outputfile
    -ip: file  --  integration -po outputfile
    -a:  out   --  alignment file, default name is alignment.txt
    -o:  out   --  phasiRNA fasta file, default name is phasiRNA.fa
    -p:  out   --  PHAS Gene fasta file; Format: >geneid/chr\tphasiRNA_cluster_region(start end)\tseq_region(start end), default name is PHAS.fa

```

```
    # options with default value
    -pl: int   --  phase length, 21 | 24, default=21
    -m:  float  --  the number for reducing the size of Y-axis. default=10

    # optional options
    -c:  file  --  reference transcritome sequence, fasta file, enable cdna based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
    -g:  file  --  reference genome sequence, fasta file, enable gdna based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
    -f:  file  --  full length transcriptome sequence, fasta file, enable flnc based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
    -pc: str  --  plot cdna based phasiRNA cluster, y | n, defaut=y
    -pg: str  --  plot gdna based phasiRNA cluster, y | n, defaut=y
    -pf: str  --  plot flnc based phasiRNA cluster, y | n, defaut=y

    # other
    -v:        --  print version information
    -h:        --  print help information
```

5. Initiator prediction and verification

```
phasiHunter target -q <osa_miRNA.fa> -b <SRR5049781_PHAS.fa> -o <SRR5049781_miR.txt> -t

phasiHunter initiator -i <SRR5049781_phasiRNA_dup.txt> -j <SRR5049781_miR.txt> -ip <SRR5049781_phas.txt> -o <SRR5049781_initiator.txt>

phasiHunter deg -i <degradome_PHAS.map> -q <osa_miRNA.fa> -j <SRR5049781_initiator.txt> -t <SRR5049781_PHAS.fa> -o <SRR5049781_initiator_verified.txt> -in <y>
```

- target module usage

```
Usage:
    perl /home/user/volumes/PhasiHunter/bin/TarHunterL_Modified.pl -q <mir_file> -b <targ_file> -o <out_file> [Options]

Required arguments:
    -q (--qmir):         query miRNA file
    -b (--targ):         target file
    -o (--output):       output file

Options:

    -M (--total_misp):   max. total mispairs              [Default: off]
    -m (--seed_misp):    max. seed mispairs               [Default: off]
    -f (--score):        score cutoff                     [Default:  4 ]

    -I (--mimics):       eTM search                       [Default: off]
    -i (--mimics_str):   eTM stringency
                        (0: strict, 1: relaxed)          [Default:  0 ]
```

```
    -T (--threads):      FASTA threads                    [Default:  1 ]
    -t (--tab):          tabular format output            [Default: off]
    -h (--help):         help information

Dependencies:
    fasta36
```

- initiator module usage

```
initiator option:
  -i  [str]integration -o output
  -j  [str]the target predicted by psRNAtarget server or target module
  -ip [str]integration -po output
  -pd [int]the microRNA distance away to phase border, default=105(21) or
120 (24), optional
  -pl [int]21 or 24, the phase length of 21 or 24, default=21
  -ps [int]0 or 1, the position of cleavage at 10(0) or 9-11 (1), default=1
  -o  [str]outputfilename.
  -h  print the version and details of the usage
```

- deg module usage

```
// function: vertified the sRNA - Target interaction with degradome data

  options:
  -i: <inputfilename>     --    mapping file for degradome data mapping
transcripts, by bowtie
  -q: <sRNA fasta>        --    small RNA sequences used for target
prediction, fasta
  -j: <inputfilename>     --    from psRNATarget batch download file or
initiator output
  -t: <inputfilename>     --    transcripts file, fasta
  -o: <outputfilename>    --    matched map file with only matched records
  -s: <shift_number>      --    if shifts=0 then cleaved exactly at pos.10,
default=1
  -m: <minum deg_num>     --    minum number of degradome reads, int,
default=0
  -p: <T-plot function>   --    enable the plot function, y | n, default='n'
  -in: <bool>             --    y | n, use initiator output information
  -pl [int]               --    1,plot only category 1; 2, plot categories 1
and 2, default=1
  -pf [str]               --    output folder name, for exporting t-plot
images and outputfile
  --lib [str]             --    library name
  -less                   --    only output cat_1 and cat_2 information

  ************************
  //About the categories:
  Cat #1, degradome read at the cleavage site is most abundant.
```

```
  Cat #2, the read is less than the most abudant one, but higher than the median.
  Cat #3, the read is less than the median, but high than 1
  Cat #4, the read is identical or less than 1 (if degradome data is normalized)
```

6. PhasiRNA target prediction and verification

```
phasiHunter target -q <SRR5049781_phasiRNA.fa> -t <oryza_sativa_cdna.fa> -o <SRR5049781_phasiRNA_target.txt>

phasiHunter deg -i <degradome_cdna.map> -q <SRR5049781_phasiRNA.fa> -j <SRR5049781_phasiRNA_target.txt> -t <oryza_sativa_cdna.fa> -o <SRR5049781_phasiRNA_target_verified.txt> -in <n>
```

Executing PhasiHunter with one-command module

One-command module usage

```
One command executing mode

Usage:
    phasiHunter run [-i] [config file]
    phasiHunter run -d

option:
    -i: yaml format config file
    -d: using the default config, defalut config file is /foo/PhasiHunter/bin/config.yaml
    -h: print help information

WARNIG: make sure choose the correct config file before run this command
```

Default config.yaml file

Some INPUT and OUTPUT still need modified when using.

```yaml
# Please provide the full path to the input file

# Configure the modules that need to be run
# y means enable, n means disable
Runing_module:
  preprocess: y
  phase: y
  integration: y
  visulization: y
  initiator_prediction_and_verification:
    target: y
    initiator: y
    deg: y
```

```yaml
  phasiRNA_target_prediction_and_verification:
    phasiRNA_target: y
    phasiRNA_deg: y

# Configure the preprocess module
preprocess:
  # raw(mode): trim adaptor --> normalization --> length and abundance filter --> mapping
  # clean(mode): normalization --> length and abundance filter --> mapping
  # mapping(mode): mapping
  mode: r # [r | c | m]

  # for r mode: fastq file or fastq.gz file
  # for c mode: fasta file or fasta.gz file
  # for m mode: length and abundance filter fasta file
  # ** INPUT **
  inputfile: /home/user/test_osa/SRR5049781.fastq.gz

  # reference sequence fasta file
  # ** INPUT **
  reference_fasta: # disable when index parameter enable, multiple sequence can provided here
    - /home/user/test_osa/oryza_sativa_cdna.fa
    - /home/user/test_osa/oryza_sativa_gdna.fa

  # index prefix, reference_fasta option will be ignored when index enable, multiple index can provided here
  # ** INPUT **
  index:
    - /home/user/test_osa/index/oryza_sativa_cdna_index
    - /home/user/test_osa/index/oryza_sativa_gdna_index

  # outfile name, relative path is work for outputfile, the number must be the same as the number of reference_fasta or indexe
  # ** OUTPUT **
  outfile_name:
    - SRR5049781_processed_cdna.map
    - SRR5049781_processed_gdna.map

  # adaptor trim parallel cores; <8 is recommend, only need in r mode
  trim_adaptor_cores: 1

  # bowtie parallel cores
  bowtie_mapping_cores: 1

  # max hits when mapping to ref sequence
  bowtie_max_hits_cutoff: 10

  # minimal sRNA reads length cutof
  minimal_sRNA_length_cutoff: 19

  # maxmial sRNA reads length cutoff
  maxmial_sRNA_length_cutoff: 25
```

```yaml

  # sRNA reads cpm cutoff
  sRNA_expression_cutoff: 1

  # normalization base
  library_normalization_base: 1000000


# Configure the phase module
phase:
  # map file based on reference transcriptome sequence
  # ** INPUT **
  mapped_cdna_file: /home/user/test_osa/SRR5049781_processed_cdna.map

  # map file based on reference genome sequence
  # ** INPUT **
  mapped_gdna_file: /home/user/test_osa/SRR5049781_processed_gdna.map

  # map file based on full length transcriptome sequence
  # ** INPUT **
  mapped_flnc_file:

  # reference transcritome sequence, fasta file
  # ** INPUT **
  cdna_fasta: /home/user/test_osa/oryza_sativa_cdna.fa

  # reference genome sequence, fasta file
  # ** INPUT **
  gdna_fasta: /home/user/test_osa/oryza_sativa_gdna.fa

  # full length transcriptome sequence, fasta file
  # ** INPUT **
  flnc_fasta:

  # sRNA file
  # ** INPUT **
  sRNA_fa: /home/user/test_osa/SRR5049781_trimmed_format_filter.fa

  # allsiRNA cluster output
  # ** OUTPUT **
  allsiRNA_cluster_output: phase_a.txt

  # phasiRNA cluster output file
  # ** OUTPUT **
  phasiRNA_cluster_output: phase_o.txt

  # phasiRNA prediction method, h(hypergeometric test) | p(phase score) | b
(both)
  phasiRNA_prediction_method: b

  # phasiRNA cluster island
  phasiRNA_cluster_island: 5

```

```yaml
    # phase length
    phase_length: 21

    # phase number
    phase_number_cutoff: 4

    # max hits when mapping to ref sequence
    bowtie_max_hits_cutoff: 10

    # parallel number
    parallel_cores: 20

    # pvalue cutoff, only function with h/b method applied
    pvalue_cutoff: 0.001

    # phase score cutoff, only function with p/b method applied
    phase_score_cutoff: 15

    # phase ratio cutoff, only function with p/b method applied
    phase_ratio_cutoff: 0.4

    # delete .phasiHuter_bowtieIndex, y|n
    delete_index: y


  # Configure the integration module
  integration:
    # phase module phasiRNA_cluster_output
    # ** INPUT **
    o_inputfile: /home/user/test_osa/phase_o.txt

    # phase module allsiRNA_cluster_output
    # ** INPUT **
    a_inputfile: /home/user/test_osa/phase_a.txt

    # reference genome gff3 file
    # ** INPUT **
    gff3: /home/user/test_osa/oryza_sativa_gdna.gff3

    # y | n, whether exist gdna based PHAS loci
    gdna_based_PHAS_loci: y

    # integration phasiRNA cluster
    # ** OUTPUT **
    integration_phasiRNA_cluster: integration_o.txt

    # integration all siRNA cluste
    # ** OUTPUT **
    integration_allsiRNA_cluster: integration_a.txt

    # integration summary
    # ** OUTPUT **
    integration_summary: integration_s.txt
```

```
168
169     # PHAS loci information
170     # ** OUTPUT **
171     integration_PHAS_loci_info: integration_p.txt
172
173     # parallel number
174     parallel_cores: 1
175
176     # phase number
177     phase_number_cutoff: 4
178
179     # phase length
180     phase_length: 21
181
182     # pvalue cutoff
183     pvalue_cutoff: 0.001
184
185     # phasiRNA cluster island
186     phasiRNA_cluster_island: 5
187
188     # y | n, discard only P method result
189     discard_only_P_method_result: y
190
191     # full length transcript annotation file
192     flnc_annotation_file:
193
194
195   # Configure the visulization module
196   visulization:
197     # integration module integration_phasiRNA_cluster
198     # ** INPUT **
199     o_inputfile: /home/user/test_osa/integration_o.txt
200
201     # integration module integration_allsiRNA_cluster
202     # ** INPUT **
203     a_inputfile: /home/user/test_osa/integration_a.txt
204
205     # integration integration_PHAS_loci_info
206     # ** INPUT **
207     p_inputfile: /home/user/test_osa/integration_p.txt
208
209     # alignment file
210     # ** OUTPUT **
211     output_alignment_file: alignment.txt
212
213     # phasiRNA fasta file
214     # ** OUTPUT **
215     output_phasiRNA_fa: phasiRNA.fa
216
217     # PHAS Gene fasta file, Format:
    >geneid/chr\tphasiRNA_cluster_region(start end)\tseq_region(start end)
218     # ** OUTPUT **
219     output_PHAS_fa: PHAS.fa
```

```
220
221   # phase length
222   phase_length: 21
223
224   # the number for reducing the size of Y-axis
225   Y_axis: 10
226
227   # reference transcritome sequence, fasta file, enable cdna based
    phasiRNA.fa, PHAS.fa, Alignmen, Plot output
228   # ** INPUT **
229   cdna_fasta: /home/user/test_osa/oryza_sativa_cdna.fa
230
231   # reference genome sequence, fasta file, enable gdna based phasiRNA.fa,
    PHAS.fa, Alignmen, Plot output
232   # ** INPUT **
233   gdna_fasta: /home/user/test_osa/oryza_sativa_gdna.fa
234
235   # full length transcriptome sequence, fasta file, enable flnc based
    phasiRNA.fa, PHAS.fa, Alignmen, Plot output
236   # ** INPUT **
237   flnc_fasta:
238
239   # plot cdna based phasiRNA cluster, y | n
240   plot_cdna_based_phasiRNA_cluster: y
241
242   # plot gdna based phasiRNA cluster, y | n
243   plot_gdna_based_phasiRNA_cluster: y
244
245   # plot flnc based phasiRNA cluster, y | n
246   plot_flnc_based_phasiRNA_cluster: n
247
248
249 # Configure the target module
250 target:
251   # query miRNA file, fasta format
252   # ** INPUT **
253   query_fa: /home/user/test_osa/osa.miRbase.fa
254
255   # PHAS.fa/transcript.fa, fasta file
256   # ** INPUT **
257   subject_fa: /home/user/test_osa/PHAS.fa
258
259   # output file
260   # ** OUTPUT **
261   output: miR_target.txt
262
263   # max. total mispairs
264   total_misp: off
265
266   # max. seed mispairs
267   seed_misp: off
268
269   # score cutoff
```

```
    score: 4

    # eTM search
    mimics: off

    # eTM stringency, (0: strict, 1: relaxed)
    mimics_str: 0

    # fasta36 threads
    threads: 10


  # Configure the initiator module
  initiator:
    # integration module integration_phasiRNA_cluster
    # ** INPUT **
    i_input_file: /home/user/test_osa/integration_o.txt

    # the target predicted by psRNAtarget server or target module
    # ** INPUT **
    j_input_file: /home/user/test_osa/miR_target.txt

    # integration module integration_PHAS_loci_info
    # ** INPUT **
    p_input_file: /home/user/test_osa/integration_p.txt

    # the microRNA distance away to phase border, default=105(21) or 120 (24)
    sRNA_distance: 5

    # 21 or 24, the phase length of 21 or 24,
    phase_length: 21

    # 0 or 1, the position of cleavage at 10(0) or 9-11 (1)
    cleavage_shift: 1

    # outputfilename
    # ** OUTPUT **
    outputfile: initiator.txt


  # Configure the deg module
  deg:
    # mapping file for degradome data mapping transcripts, by bowtie
    # ** INPUT **
    inputfile:
      - /home/user/test_osa/deg/GSM1040649_format_filter.map
      - /home/user/test_osa/deg/GSM1040650_format_filter.map

    # miRNA sequences used for target prediction, fasta
    # ** INPUT **
    query_fa: /home/user/test_osa/osa.miRbase.fa

    # initiator module outputfile
```

```yaml
    # ** INPUT **
    STI_result: /home/user/test_osa/initiator.txt

    # transcripts file, fasta
    # ** INPUT **
    transcript_fa: /home/user/test_osa/oryza_sativa_cdna.fa

    # matched map file with only matched records
    # ** OUTPUT **
    output:
      - GSM1040649_MTI_deg.txt
      - GSM1040650_MTI_deg.txt

    # if shifts=0 then cleaved exactly at pos.10
    shift: 1

    # minum number of degradome reads, int
    minum_deg_abun: 0

    # enable the plot function, y | n
    T_plot: y

    # y | n, use initiator output information
    initiator: y

    # 1,plot only category 1; 2, plot categories 1 and 2
    plot_categories: 1

    # output folder name, for exporting t-plot images and outputfile
    plot_folder: MTI_deg

    # library name
    library:
      - GSM1040649
      - GSM1040650

    # only output cat_1 and cat_2 information
    less: y


  # Configure the phasiRNA_target module
  phasiRNA_target:
    # query phasiRNA file, fasta format
    # ** INPUT **
    query_fa: /home/user/test_osa/phasiRNA.fa

    # target file, fasta file
    # ** INPUT **
    subject_fa: /home/user/test_osa/oryza_sativa_cdna.fa

    # output file
    # ** OUTPUT **
    output: phasiRNA_target.txt
```

```
376
377     # max. total mispairs
378     total_misp: off
379
380     # max. seed mispairs
381     seed_misp: off
382
383     # score cutoff
384     score: 4
385
386     # eTM search
387     mimics: off
388
389     # eTM stringency, (0: strict, 1: relaxed)
390     mimics_str: 0
391
392     # fasta36 threads
393     threads: 10
394
395
396   # Configure the phasiRNA_deg module
397   phasiRNA_deg:
398     # mapping file for degradome data mapping transcripts, by bowtie
399     # ** INPUT **
400     inputfile:
401       - /home/user/test_osa/deg/GSM1040649_format_filter.map
402       - /home/user/test_osa/deg/GSM1040650_format_filter.map
403
404     # phasiRNA sequences used for target prediction, fasta
405     # ** INPUT **
406     query_fa: /home/user/test_osa/phasiRNA.fa
407
408     # psRNATarget/target outputfile
409     # ** INPUT **
410     STI_result: /home/user/test_osa/phasiRNA_target.txt
411
412     # transcripts file, fasta
413     # ** INPUT **
414     transcript_fa: /home/user/test_osa/oryza_sativa_cdna.fa
415
416     # matched map file with only matched records
417     # ** OUTPUT **
418     output:
419       - GSM1040649_PTI_deg.txt
420       - GSM1040650_PTI_deg.txt
421
422     # if shifts=0 then cleaved exactly at pos.10
423     shift: 1
424
425     # minum number of degradome reads, int
426     minum_deg_abun: 0
427
428     # enable the plot function, y | n
```

```
  T_plot: y

  # y | n, use initiator output information, for phasiRNA_deg, it must be n
  initiator: n

  # 1,plot only category 1; 2, plot categories 1 and 2
  plot_categories: 1

  # output folder name, for exporting t-plot images and outputfile
  plot_folder: PTI_deg

  # library name
  library:
    - GSM1040649
    - GSM1040650

  # only output cat_1 and cat_2 information
  less: y
```

The main output file

- preprocess module
  - preprocessed fasta file
  - alignment file generated by bowtie
- phase module
  - redundant allsiRNA cluster output
  - redundant phasiRNA cluster output
- integration module
  - integrated PHAS loci information
  - integration summary information
  - integrated allsiRNA cluster output
  - integrated phasiRNA cluster output
- visulization module
  - phasiRNA fasta file
  - PHAS loci fasta file
  - phasiRNA alignment result
  - phasiRNA cluster plot
- initiator_prediction_and_verification
  - miRNA-PHAS_loci interaction output
  - Predicted phase initiator output
  - vertified phase initiator output with degradome data
  - degradome verification t-plot
- phasiRNA_target_prediction_and_verification
  - phasiRNA-target interaction output
  - vertified phasiRNA-target interaction with degradome data
  - degradome verification t-plot

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