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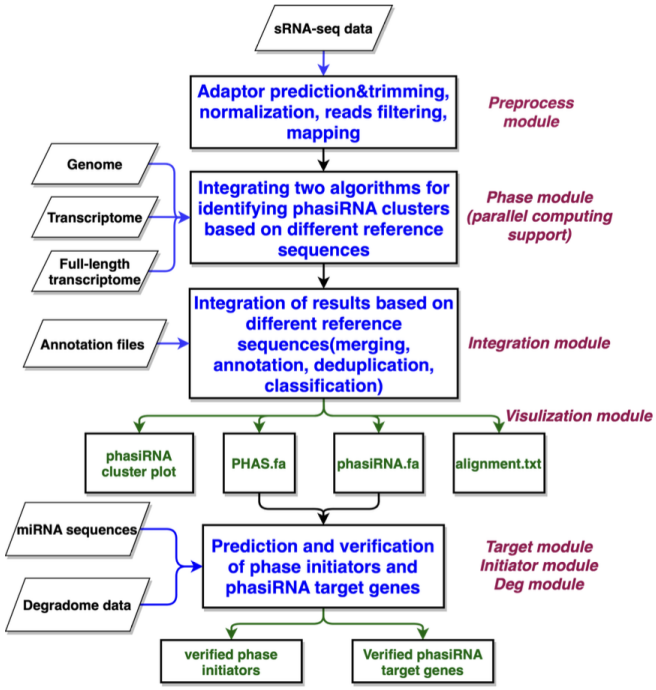
User guide

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)

Installation

Manual Installation

1. Install all dependencies
2. Clone phasihunter

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

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

   example:

   `echo "export PATH=\$PATH:/home/user/volumes/PhasiHunter >> ~/.bashrc"`
4. 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
```

Docker image

For convenience, we also provide a Docker image at

`https://hub.docker.com/repository/docker/zacksfeng/phasihunter`

The Docker image has been configured with all the dependencies required for running phasiHunter.

Conda/mamba configure file

We also provide a conda/mamba environment configuration file. User can install all the required dependencies with command `conda/mamba install -f /foo/PhasiHunter/bin/env.yaml`

Demo data

Download link

`https://cbi.njau.edu.cn/PhasiHunter_demo_data/test_osa.tar.gz`

Executing PhasiHunter with step-by-step submodules.

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

1. Data pre-process

```
phasiHunter preprocess -m r -i /home/user/test_osa/SRR5049781.fastq.gz -mi 19 -ma 25 -e 1 -n 1000000 -o /home/user/test_osa/SRR5049781_processed_cdna.map -in /home/user/test_osa/index/oryza_sativa_cdna_index

phasiHunter preprocess -m m -i /home/user/test_osa/SRR5049781_trimmed_format_filter.fa -mi 19 -ma 25 -e 1 -n 1000000 -o /home/user/test_osa/SRR5049781_processed_gdna.map -in /home/user/test_osa/index/oryza_sativa_gdna_index
```

- 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 /home/user/test_osa/SRR5049781_processed_cdna.map -c
/home/user/test_osa/oryza_sativa_cdna.fa -gm
/home/user/test_osa/SRR5049781_processed_gdna.map -g
/home/user/test_osa/oryza_sativa_gdna.fa -fm None -f None -fa
/home/user/test_osa/SRR5049781_trimmed_format_filter.fa -a
/home/user/test_osa/phase_a.txt -o /home/user/test_osa/phase_o.txt -me b -il
5 -pl 21 -pn 4 -mh 10 -j 20 -pv 0.001 -ps 15 -pr 0.4 -cl y
```

- 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 /home/user/test_osa/phase_o.txt -ia
/home/user/test_osa/phase_a.txt -an
/home/user/test_osa/oryza_sativa_gdna.gff3 -g y -o
/home/user/test_osa/integration_o.txt -a
/home/user/test_osa/integration_a.txt -s
/home/user/test_osa/integration_s.txt -po
/home/user/test_osa/integration_p.txt -j 1 -pn 4 -pl 21 -pv 0.001 -il 5
```

- 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 /home/user/test_osa/integration_o.txt -ia
/home/user/test_osa/integration_a.txt -ip
/home/user/test_osa/integration_p.txt -a /home/user/test_osa/alignment.txt -
o /home/user/test_osa/phasiRNA.fa -p /home/user/test_osa/PHAS.fa -pl 21 -m
10 -c /home/user/test_osa/oryza_sativa_cdna.fa -g
/home/user/test_osa/oryza_sativa_gdna.fa -f None -pc y -pg y -pf n
```

- 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 /home/user/test_osa/osa.miRbase.fa -b
/home/user/test_osa/PHAS.fa -o /home/user/test_osa/miR_target.txt -T 10

phasiHunter initiator -i /home/user/test_osa/integration_o.txt -j
/home/user/test_osa/miR_target.txt -ip /home/user/test_osa/integration_p.txt
-pd 5 -pl 21 -ps 1 -o /home/user/test_osa/initiator.txt

phasiHunter deg -i /home/user/test_osa/deg/GSM1040649_format_filter.map -q
/home/user/test_osa/osa.miRbase.fa -j /home/user/test_osa/initiator.txt -t
/home/user/test_osa/oryza_sativa_cdna.fa -o GSM1040649_MTI_deg.txt -s 1 -m 0
-p y -in y -pl 1 -pf MTI_deg --lib GSM1040649 -less
```

- 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 /home/user/test_osa/phasiRNA.fa -b /home/user/test_osa/oryza_sativa_cdna.fa -o /home/user/test_osa/phasiRNA_target.txt -T 10

phasiHunter deg -i /home/user/test_osa/deg/GSM1040649_format_filter.map -q /home/user/test_osa/phasiRNA.fa -j /home/user/test_osa/phasiRNA_target.txt -t /home/user/test_osa/oryza_sativa_cdna.fa -o GSM1040649_PTI_deg.txt -s 1 -m 0 -p y -in n -pl 1 -pf PTI_deg --lib GSM1040649 -less
```

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
  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, but absolute path is still recommended. The number must be the same as the number of reference_fasta or indexs
```

```
  # ** OUTPUT **
  outfile_name:
    - /home/user/test_osa/SRR5049781_processed_cdna.map
    - /home/user/test_osa/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

  # sRNA reads cpm cutoff
  sRNA_expression_cutoff: 1

  # normalization base
  library_normalization_base: 1000000


# Configure the phase module
# predicting with only one reference sequence or multiple reference
sequences
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 **
```

```yaml
  flnc_fasta:

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

  # allsiRNA cluster output
  # ** OUTPUT **
  allsiRNA_cluster_output: /home/user/test_osa/phase_a.txt

  # phasiRNA cluster output file
  # ** OUTPUT **
  phasiRNA_cluster_output: /home/user/test_osa/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

  # 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: /home/user/test_osa/integration_o.txt

    # integration all siRNA cluste
    # ** OUTPUT **
    integration_allsiRNA_cluster: /home/user/test_osa/integration_a.txt

    # integration summary
    # ** OUTPUT **
    integration_summary: /home/user/test_osa/integration_s.txt

    # PHAS Loci information
    # ** OUTPUT **
    integration_PHAS_Loci_info: /home/user/test_osa/integration_p.txt

    # parallel number
    parallel_cores: 1

    # phase number
    phase_number_cutoff: 4

    # phase length
    phase_length: 21

    # pvalue cutoff
    pvalue_cutoff: 0.001

    # phasiRNA cluster island
    phasiRNA_cluster_island: 5

    # y | n, discard only P method result
    discard_only_P_method_result: y

    # full length transcript annotation file
    flnc_annotation_file:


  # Configure the visulization module
  visulization:
    # integration module integration_phasiRNA_cluster
    # ** INPUT **
    o_inputfile: /home/user/test_osa/integration_o.txt
```

```
  # integration module integration_allsiRNA_cluster
  # ** INPUT **
  a_inputfile: /home/user/test_osa/integration_a.txt

  # integration integration_PHAS_Loci_info
  # ** INPUT **
  p_inputfile: /home/user/test_osa/integration_p.txt

  # alignment file
  # ** OUTPUT **
  output_alignment_file: /home/user/test_osa/alignment.txt

  # phasiRNA fasta file
  # ** OUTPUT **
  output_phasiRNA_fa: /home/user/test_osa/phasiRNA.fa

  # PHAS Gene fasta file, Format: >geneid/chr\tphasiRNA_cluster_region(start end)\tseq_region(start end)
  # ** OUTPUT **
  output_PHAS_fa: /home/user/test_osa/PHAS.fa

  # phase length
  phase_length: 21

  # the number for reducing the size of Y-axis
  Y_axis: 10

  # reference transcritome sequence, fasta file, enable cdna based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
  # ** INPUT **
  cdna_fasta: /home/user/test_osa/oryza_sativa_cdna.fa

  # reference genome sequence, fasta file, enable gdna based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
  # ** INPUT **
  gdna_fasta: /home/user/test_osa/oryza_sativa_gdna.fa

  # full length transcriptome sequence, fasta file, enable flnc based phasiRNA.fa, PHAS.fa, Alignmen, Plot output
  # ** INPUT **
  flnc_fasta:

  # plot cdna based phasiRNA cluster, y | n
  plot_cdna_based_phasiRNA_cluster: y

  # plot gdna based phasiRNA cluster, y | n
  plot_gdna_based_phasiRNA_cluster: y

  # plot flnc based phasiRNA cluster, y | n
  plot_flnc_based_phasiRNA_cluster: n
```

```yaml
# Configure the target module
target:
  # query miRNA file, fasta format
  # ** INPUT **
  query_fa: /home/user/test_osa/osa.miRbase.fa

  # PHAS.fa/transcript.fa, fasta file
  # ** INPUT **
  subject_fa: /home/user/test_osa/PHAS.fa

  # output file
  # ** OUTPUT **
  output: /home/user/test_osa/miR_target.txt

  # max. total mispairs
  total_misp: off

  # max. seed mispairs
  seed_misp: off

  # 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: /home/user/test_osa/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
    # ** 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
    # filename only, do not input directory
    # ** 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: /home/user/test_osa/phasiRNA_target.txt

  # max. total mispairs
  total_misp: off

  # max. seed mispairs
  seed_misp: off

  # score cutoff
  score: 4

  # eTM search
  mimics: off

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

  # fasta36 threads
  threads: 10


# Configure the phasiRNA_deg module
phasiRNA_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

  # phasiRNA sequences used for target prediction, fasta
  # ** INPUT **
  query_fa: /home/user/test_osa/phasiRNA.fa
```

```
409
410     # psRNATarget/target outputfile
411     # ** INPUT **
412     STI_result: /home/user/test_osa/phasiRNA_target.txt
413
414     # transcripts file, fasta
415     # ** INPUT **
416     transcript_fa: /home/user/test_osa/oryza_sativa_cdna.fa
417
418     # matched map file with only matched records
419     # filename only, do not input directory
420     # ** OUTPUT **
421     output:
422       - GSM1040649_PTI_deg.txt
423       - GSM1040650_PTI_deg.txt
424
425     # if shifts=0 then cleaved exactly at pos.10
426     shift: 1
427
428     # minum number of degradome reads, int
429     minum_deg_abun: 0
430
431     # enable the plot function, y | n
432     T_plot: y
433
434     # y | n, use initiator output information, for phasiRNA_deg, it must be n
435     initiator: n
436
437     # 1,plot only category 1; 2, plot categories 1 and 2
438     plot_categories: 1
439
440     # output folder name, for exporting t-plot images and outputfile
441     plot_folder: PTI_deg
442
443     # library name
444     library:
445       - GSM1040649
446       - GSM1040650
447
448     # only output cat_1 and cat_2 information
449     less: y
```

The main output file

- preprocess module
  - preprocessed fasta file
  - alignment file generated by bowtie
- phase module
  - redundant allsiRNA cluster output
    - table header: gene, strand, sRNA_position, sRNA_abundance, sRNA_record, sRNA_sequence, sRNA_length, pvalue, phase_ratio, phase_number,

phase_abundance, phase_score, marker

    - redundant phasiRNA cluster output
        - table header: PHAS_gene, strand, phasiRNA_position, phasiRNA_abundance, phasiRNA_record, phasiRNA_sequence, phasiRNA_length, pvalue, phase_ratio, phase_number, phase_abundance, phase_score, marker
- integration module
    - integrated PHAS loci information
    - integration summary information
    - integrated allsiRNA cluster output
        - table header: gene, strand, sRNA_position, sRNA_abundance, sRNA_record, sRNA_sequence, sRNA_length, phase_ratio, phase_number, phase_abundance, phase_score, pvalue, gene_annotation, marker
    - integrated phasiRNA cluster output
        - table header: PHAS_gene, strand, phasiRNA_position, phasiRNA_abundance, phasiRNA_record, phasiRNA_sequence, phasiRNA_length, phase_ratio, phase_number, phase_abundance, phase_score, pvalue, PHAS_gene_annotation, marker
- visulization module
    - phasiRNA fasta file
        - id description: recorder__PHAS_gene__position__abundance_strand_order
    - PHAS loci fasta file
        - id description: recorder__PHAS_gene__[start]__[end]__[extend_start]__[extend_end]__[marker]
    - 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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