

Manual for tsRFinder

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Abstract

The tsRFinder is a lightweight, fast and reliable tool for prediction and annotation of tRNA-derived small RNAs using next-generation sequencing data. It's a free open source software available at <https://github.com/wangqinhu/tsRFinder>.

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1 Introduction

Small RNAs, such as miRNA, siRNA, and piRNA, are key regulators of gene expression. The tRNA-derived small RNA (tsRNA) is a recently identified novel class of small RNA, no public tool has tailored for tsRNA analysis yet. We thus developed tsRFinder for tsRNA prediction and annotation with additional sequence and statistical analysis, using small RNA sequencing data and the reference genome sequences.

2 How to install

2.1 Dependency

The tsRFinder depends on a few free open source softwares, please check and install them at first:

- Perl, v5.10.1 or higher, required for tsRFinder.pl execution. Always build-in in most of the UNIX-like OS.
<http://www.perl.org/get.html>
- R, v2.15.2 or higher, required for small RNA data analysis and illustration.
<http://www.r-project.org>
- bowtie, v1.0.0 or higher, required for small RNA mapping.
<https://github.com/BenLangmead/bowtie>
- tRNAscan-SE, v1.3.1 or higher, required for tRNA prediction. It's optional if you prefer manual tRNA input.
<http://lowelab.ucsc.edu/tRNAscan-SE>

2.2 Installation

You may clone ¹ tsRFinder by typing the following in the terminal.

¹If git is not installed, download it from <http://git-scm.com>

Clone tsRFinder

```
git clone https://github.com/wangqinhu/tsRFinder.git
```

Alternatively, you may download it from the following URL.

Latest release of tsRFinder

```
https://github.com/wangqinhu/tsRFinder/releases/latest
```

tsRFinder is maintained on GitHub and is ready-to-use, no compilation is required. However, if you take 1 - 2 minutes to improve the configuration, it may save you a lot of time for trouble shooting. Once tsRFinder is cloned or unpacked, move the entire directory to a proper place (or current working directory, such as home directory) and add the tsRFinder path to your environment settings. For example, if tsRFinder is placed in "/the/path/of/tsRFinder", then type the following in the terminal if you are using bash.

Setup tsRFinder

```
echo export tsR_dir="/the/path/of/tsRFinder" >> $HOME/.bashrc  
source ~/.bashrc
```

tsRFinder is now ready for your dataset, if you want to run tsRFinder.pl as a system command, create a soft link for it:

Make a soft link for tsRFinder.pl

```
ln -s `pwd`/tsRFinder/tsRFinder.pl /usr/local/bin/
```

3 How to use

3.1 Preparing the dataset

Before running tsRFinder, you are asked to prepare/download the following two files: (1) the reference genome sequence, or the reference tRNA sequence and, (2) the small RNA reads. You are strongly recommended to use the reference genome sequence and raw small RNA sequencing data since tsRFinder is capable of preparing the reference tRNA data and clean

small RNA data automatically. In case you prefer to preparing tRNA and small RNA files manually, see "demo/tRNA.fas" and "demo/sRNA.fa.gz" or access the format description in the FAQ section.

3.2 Running the pipeline

Once tsRFinder is properly installed, you can run tsRFinder directly from terminal. A typical tsRFinder job can be easily finished on a modern MacBook (running OS X) or laptop (running Linux), typically within 10 minutes.

To know how to use tsRFinder, see the following usage:

Usage of tsRFinder: ./tsRFinder.pl -h

tsRFinder usage:

tsRFinder.pl <option>

-c Configuration file
-l Label
-g Reference genomic sequence file, conflict with -t
-t Reference tRNA sequence file, conflict with -g
-s sRNA sequence file
-a Adaptor sequence
-n Min read length [default 18]
-x Max read length [default 45]
-e Min expression level [default 10]
-u Mature tsRNA level cut-off [default 10]
-f sRNA family threshold [default 72]
-d Method for sRNA normalization [default rptm/rpm/no]
-w tRNA with/without label [default no/yes]
-o Output compressed tarball [default no/yes]
-i Interactive [default yes/no]
-m Mode, run/debug [default run/debug]
-h Help
-v Version

Examples:

tsRFinder.pl -c demo/tsR.conf

tsRFinder.pl -c demo/tsR.alt.conf

tsRFinder has two ways for arguments input, either configuration file or command line option is acceptable. We recommend you to use a command

line option for debugging and building your configuration file. Once your inputs are determined, you can write it to a configuration file for analysis. For preparation configuration file, see "demo/tsR.conf" or "Demo running" part of this manual.

To submit tsRFinder job on a cluster managed with sun grid engine (sge), use "demo/tsR.sge.sh" as a template:

```
demo/tsR.sge.sh

#!/bin/bash

#$ -S /bin/bash
#$ -cwd
#$ -N tsRFinder
#$ -V

if [ $# != 1 ] ; then
echo
echo "Usage: $0 config_file"
echo " e.g.: $0 tsR.conf"
echo
exit
fi

echo -n "Running on: "
hostname
echo "SGE job id: $JOB_ID"
echo -n "Begin time: "
date

echo
echo "||||||||||||||||||||||||||||||||||||||||"
echo

tsRFinder.pl -i no -o yes -c $1

echo
echo "||||||||||||||||||||||||||||||||||||||||"
echo

echo -n "End time: "
date
```

For example, you can submit the demo tsRFinder job like this:

```
submit a demo tsRFinder job

qsub demo/tsR.sge.sh demo/tsR.conf
```

4 Demo

To have a quick but rough overview that how tsRFinder looks like, see the animated gif demo in "doc/demo.gif" or online.

4.1 Demo data

Demo refseq: we used several random sequences embedded with some real tRNAs as pseudo reference sequences, in FASTA format and accessible from file "demo/genome.fa". A reference genome sequence is also applicable. Figure 1 shows a refseq file in FASTA format.

```

1 >chr1
2 TTTGCTAGTCGTCATTCTAGTCTGACTGACTGCTGCTGCTGCTGACGTACGTACGACG
3 TACTTCTCGTTAGTATAGTGGTtAGTATACCCGCCTGTCACGCGGGTGACCCGGGTTCAA
4 TTCCCGGACGGGAAGGTACGTCTGTGTCTACACACTACTACTTGACTGACGTACGTACG
5 TACGTACTGACGTACGTACGTACTGTGTTGTGTGTATCGTAGTCGTAGTATCGCGTG
6 TGTGTGTACGTAGCGTGTGACACGTAGTCATCGTGACACTACTACTGTACGTACG
7 TACGTACTGA
8 >chr2
9 GTACTACTTAGTCGTCGTCAGTCAGTCAGTACGTACGTACGTACGTACGTACTACTGTAC
10 GTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGACG
11 TACGTACGTACTGACGTACGTACGTACGTACGTACGCGCCGTTGGTTTAGTGGAATATCTC
12 GTTGCCAACGAGATGCCCCGGGTTTCGATTCCCGGACGGCGCAGTCTGACTACTACTGTAC
13 TGGTACTGACGTACGTACGTACGTAGTACTGACGTACGTACTACTGTACTGACGCGCCG
14 TTGGTCTAATGGTtAGGATTCGTCCCTCCCACGGACGAGGTCTGGGGTTTCGATCCCCGGC
15 GCGCATCAGTCAGTCAACACGTACGTACGTACTGACTGACTGACTGACGTACGTACTGA
NORMAL genome.fa unix < utf-8 < fasta 4% 1:1

```

Figure 1: Screenshot of the reference sequence in FASTA format

Demo sRNA: we extracted a bit of raw reads from some experimental data as a demo. Each read of the raw small RNA data have 4 lines. Beginning with "@" identifier line, followed by sequence line, "+" identifier optional line and quality score line, as shown in Figure 2.

4.2 Demo running

tsRFinder allows you specify inputs via a separate configuration file, as shown below for an example of the content of demo tsR.conf:

```

1 @HWI-ST1106:652:H0Y8BADXX:2:1101:2120:2141 1:N:0:ATGTCA
2 GAGGAGGATGACCTTGGGCTGAGAATGGAATTCTCGGGTGCCAAGGAACT
3 +
4 CCCCCFFHHHHHJJDDGGHIBGIJJICHIIJGHIIJGIJJIIJEGIIIG
5 @HWI-ST1106:652:H0Y8BADXX:2:1101:2016:2163 1:N:0:ATGTCA
6 TGAGCTACGTGGAAGACATCGTCAGGTGGAATTCTCGGGTGCCAAGGAACT
7 +
8 CCCDFADFHHHHHJGCEEHHE@GHIJ?FHIJJIIJJJJ2@AHIIGGIGB
9 @HWI-ST1106:652:H0Y8BADXX:2:1101:2346:2189 1:N:0:ATGTCA
10 TTAATATTCCTGAACCGAGACGTGGAATTCTCGGGTGCCAAGGAACTCCA
11 +
12 ???D;DD:D4D?DEIE1)3)@FFE@1@DACB9?;DDDD)?B#####
13 @HWI-ST1106:652:H0Y8BADXX:2:1101:2392:2203 1:N:0:ATGTCA
14 TCGCGCGATTGAGCGCATTCGAGCCTGGAATTCTCGGGTGCCAAGGAACT
15 +
16 @@@DDDDD:??FHGGHIIIGDHEHEDD9(=CGGGGHIGG<EHHHBB@DCC
17 @HWI-ST1106:652:H0Y8BADXX:2:1101:2588:2161 1:N:0:ATGTCA
18 AACGAGATGCCCCGGGTTCGATTCCCGGACGGCGCACCATGGAATTCTCG
19 +

```

NORMAL sRNA.fq unix < utf-8 < fastq 0% 1:1

Figure 2: Screenshot of the small RNA sequence in FASTQ format

```

Demo configuration file for tsRFinder: demo/tsR.conf

label                :   Abc
reference_genome      :   demo/genome.fa
reference_tRNA        :
sRNA                  :   demo/sRNA.fq.gz
adaptor               :   TGGAATTCTCGGGTGCCAAGG
min_read_length       :   18
max_read_length       :   45
min_expression_level  :   10
mature_cut_off        :   10
family_threshold      :   72
tRNA_with_label       :   no
output_compressed     :   no
interactive           :   yes

```

Currently we have 14 items (18 options in command line) for configuration file filling. The argument items and the inputs are separated by colon (":"). You are recommended to use the first three letters of the investigated organism as a label (e.g. *Ath* for *Arabidopsis thaliana*, *-l* in command line option); the paths of reference genome (*-g*) and small RNA (*-s*) should be

supplied at least (tsRFinder supports both plain ASCII text and gzipped input files for sRNA and reference genome). If raw sequence data is used, the adaptor sequence (*-a*) is required. In case a reference tRNA (*-t*) is not used, leave the argument to EMPTY.

Once the configuration file (*-c*) is prepared, an analysis protocol have been determined. In the demo, the configuration file is at demo/tsR.conf, typing the following in the terminal to run tsRFinder:

Running tsRFinder demo

```
./tsRFinder.pl -c demo/tsR.conf
```

To run alternative tRNA and sRNA dataset prepared yourself, typing the following:

Running tsRFinder alternative demo

```
./tsRFinder.pl -c demo/tsR.alt.conf
```

tsRFinder allows you specify the minimum (*-n*) and maximum (*-x*) length of sequence (between 15-50 nt) for processing, you may specify the minimum expression level (*-e*) of the reads to increase reliability, as well as cut-off (*-u*) of the expression level of mature tsRNA. In case the family members are loose, the users may increase the tsRNA family threshold (*-f*) to tune it. To disable family assignment function, use a positive integer below 50. Any 0 value in the options will be replaced by the default value, if have. To make small RNAs from different samples comparable, you may use "rptm" (reads per ten millions) or "rpm" (reads per millions) method for small RNA data normalization (*-d*).

Note the minimum (*-n*), maximum (*-x*) read length, minimum expression (*-e*) level, mature (*-u*) tsRNA expression level and family (*-f*) threshold require postive number integers as inputs, filling by 0 will be replaced by the default values. The minimum and maximum read length shoud between 15 and 50 nt, threshold for sRNA family below 50 will switch off the sRNA classification function.

tsRFinder can work in run and debug modes (*-m*). In case of accessing some problems or some temporary files, you may enable the "debug" mode, otherwise use the "run" mode (default). To switch between interactive and

non-interactive mode, set option *-i* to "yes" or "no". To create a compressed tarball of the output file, use *-o yes* option please. To check the usage or version, you can use *-h* and *-v* option, respectively.

4.3 Demo output

Shown below is a demo summary:

tsRFinder demo output list

```
-----  
SUMMARY  
-----  
  
*      tRNA seq : Abc/tRNA.fa  
      Total : 5  
*      sRNA reads : Abc/sRNA.fa  
      Total : 10005215  
      Unique : 7227  
*      tRNA reads : Abc/tRNA.read.fa  
      Total : 236122  
      Unique : 50  
*      tsRNA seq : Abc/tsRNA.seq  
      Total : 7  
      Unique : 6  
* tsRNA report : Abc/tsRNA.report.xls  
  tsR-5p total : 5  
  tsR-3p total : 2  
  tsR-5p unique : 4  
  tsR-3p unique : 2  
*      Text map : Abc/tsRNA.tmap  
*      Visual map : Abc/images  
* Distribution : Abc/distribution.pdf  
*      Cleavage :  
      Detail : Abc/cleavage.txt  
      Profile : Abc/cleavage_profile.pdf  
* tsRNA family : Abc/tsRNA.fam  
* Stat. by BDI :  
  Sensitivity : 0.9593  
  Specificity : 0.7825  
  Accuracy : 0.8819
```

By default, tsRFinder delivers a summary files with some basic statistics.

The predicted or user applied tRNA sequence, the small RNA clean data, the tRNA reads, and the predicted tsRNA sequences are listed. A figure showing the length distributions of small RNA and tRNA reads (Figure 3) are included. Meanwhile, tsRFinder gives additional summary on tsRNA family, and tRNA/tsRNA expression levels (including 5' tsRNA, 3' tsRNA and their abundance), text map (tmap) showing mapping of small RNA to tRNA, graphics showing the expression level based on small RNA data, the cleavage sites, and the cleavage profile (Figure 4).

When compressed tarball output *-o yes* is enabled, all the output files were in the *label.tgz* for download, including the summary list.

4.4 Visualization of tmap data

To examine the map of tsRNA, a vim syntax plugin was developed for visualization. You may enable color text map by copying *lib/tmap.vim* into the vim syntax folder, and put the following line into your *.vimrc* file:

Set filetype tmap in vim

```
au BufNewFile,BufRead *.tmap setf tmap
```

Once tmap vim is installed, open the *tsRNA.tmap* file with vim to generate a color text map, as shown in Figure 5.

Visualization tsRNA.tmap

```
vim tsRNA.tmap
```

If you prefer plain text view without highlighting, open *tsRNA.tmap* with any text editors you have.

5 FAQ

1. Can tsRFinder run on Windows?

No. tsRFinder depends on tRNAscan-SE (UNIX source code) and uses the advantages of some build-in program of UNIX-like systems, for example,

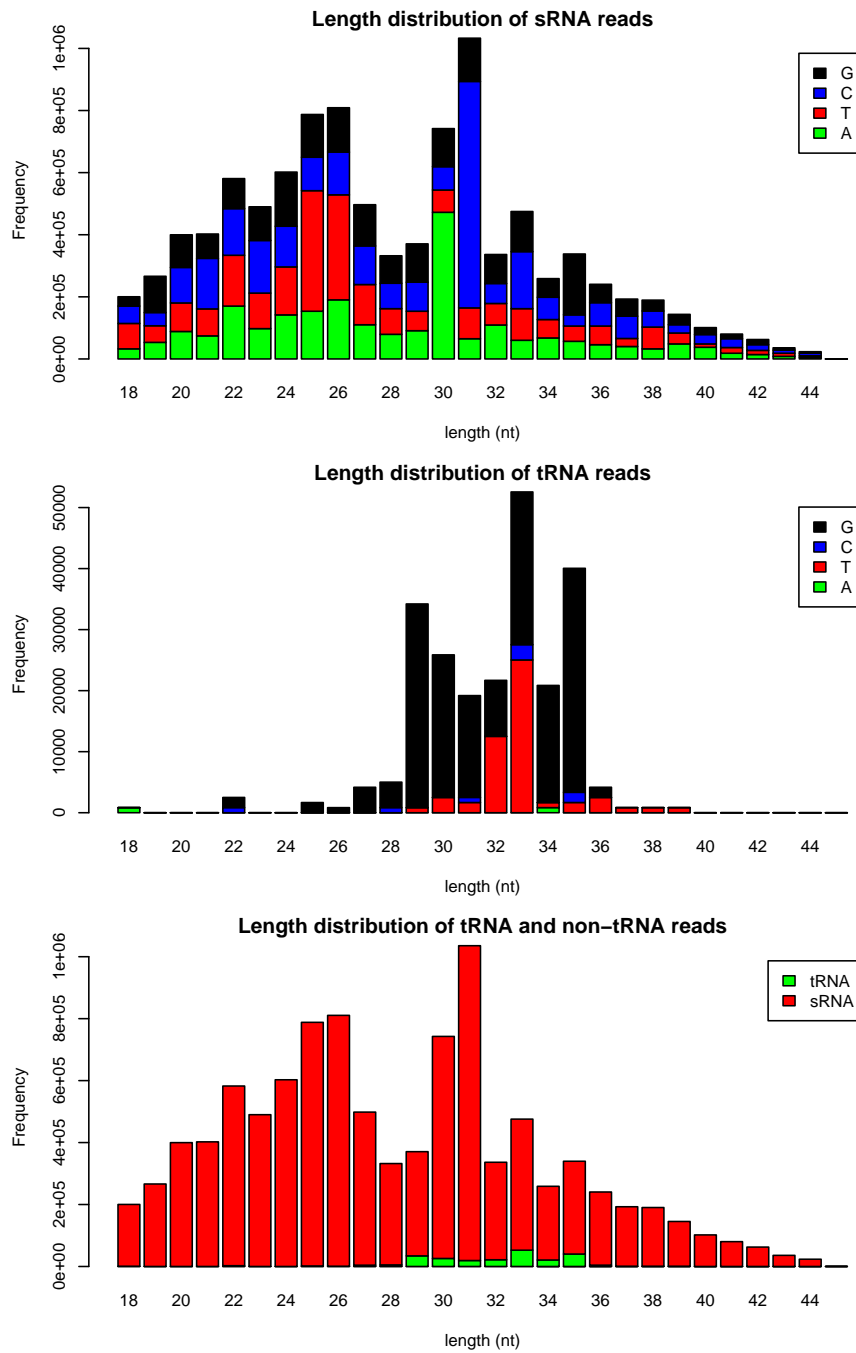


Figure 3: Length distribution of small RNA and tRNA reads

Note: The range of the small RNA length exhibited here is based on the minimum (-n) and maximum (-x) sequence length that specified in command line or the configuration file.

awk, grep and head. Running on Windows may lead unexpected errors. Linux or OS X is strongly recommended.

2. What's the length required for small RNA reads?

Ideally, sequences from 15 - 50 nt for small RNA are recommend.

3. What's the tRNA format required for input?

You are encouraged to use reference genomic sequences since tsRFinder is capable of extracting tRNA sequences via tRNAscan-SE, removing duplicated sequences, and formatting the input. In case a manually prepared file is preferred, proceed following this format: first line, begin with ">", followed by label "Abc", and the name of tRNA "tRNA - ProCGG1"; second line, the sequence of tRNA; and third line, the secondary structure of tRNA, "><" and "()" both are acceptable. Be carefully in preparing this because ">" equals "(" but not ")".

Shown below is an example.

tRNA example

```
>AbctRNA-ProCGG1
GGCCTCGTGGTCTAGTGGTATGATTCTC [NNN] AGAGGtCCCGGGTTCGATTCCCGGTGAGGCC
>>>>>>...>>>.....<<<.>>> [<.>] <<.....>>>>>>.....<<<<<<<<<<<.
```

4. What's the sRNA format required for input?

You are recommended to use raw data. In case a manually prepared sRNA file is used for input, proceed following this format: first line, begin with ">", and the label "Abc", then the 7bit index, followed by "-" (or "|", "-") and white space) and the read number; second line, the sequence of the read. One more thing, the format of sRNA data in tsRFinder is compatible with the FASTX_TOOL kit², which helps you on processing the raw sequencing data.

Shown below is an example.

sRNA example

```
>Abc0000001_772
CAGGTGGTCAGGTAGAGAATACCAAGGCGCT
>Abc0000002_475
AGGTGGTCAGGTAGAGAATACCAAGGCGCT
```

²http://hannonlab.cshl.edu/fastx_toolkit/

5. I have problems in installing tsRFinder and/or the dependencies, where can I get more help?

You can access the official support website for trouble-shooting, or open new issue for tsRFinder repository on GitHub. The URL is <https://github.com/wangqinhu/tsRFinder/issues/new>

6. Where to report bugs?

Goto <https://github.com/wangqinhu/tsRFinder/issues/new>

7. Can we use tsRFinder for commercial purpose?

Yes. tsRFinder is free, open source software, see the MIT license.

8. The 'nwalgn' not compatible to my operating system, what can I do?

tsRFinder uses Needleman-Wunsch algorithm nwalgn for small RNA alignment, with pre-building of the binaries for some of the recent OS X / Linux. If you find it not suitable for your system or want to compile it by yourself, goto lib/src directory and type 'make' to build ³ it manually.

³The gcc compiler is required. For OS X, you can install Xcode (ship with gcc)