SPORTS1.1

Small non-coding RNA annotation Pipeline Optimized for rRNA- and tRNA- Derived Small RNAs

If you use SPORTS1.1 in your work, please cite this paper:

SPORTS1.0: a tool for annotating and profiling non-coding RNAs optimized for rRNA-and tRNA-derived small RNAs

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Requirements

Linux system, enough disk space and Ram depending on the size of RNA deep sequencing data. (Tested system: ubuntu 12.04 LTS, ubuntu 16.04 LTS)

Installation

- 1. Download SPORTS1.1 pipeline package.
 - wget https://github.com/junchaoshi/SPORTS1.1/archive/master.zip
- 2. Download necessary software, packages and reference databases as listed below:
 - 1. Perl 5 (https://www.perl.org) (Tested version: v5.14.2, v5.22.1); Perl 5 might be already installed in the linux system.

- 2. Bowtie [1] (http://bowtie-bio.sourceforge.net/index.shtml) (Tested version: 1.1.2, 1.2.1.1)
- 3. SRA Toolkit (https://ncbi.github.io/sra-tools/) (Tested version: 2.8.2)
- 4. cutadapt [2] (http://cutadapt.readthedocs.io/en/stable/index.html) (Tested version: 1.11)
- 5. R (https://www.r-project.org/) (Tested version: 3.2.3, 3.2.5)
- 6. Reference database (See lists and download link of all pre-compiled species' databases in Pre-compiled Databases Instruction)
- 3. Installation tutorial for software and packages.
 - 1. Install SPORTS1.1
 - 1. Unpack SPORTS1.1 package.

```
unzip SPORTS1.1-master.zip
```

2. Attach the SPORTS directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_SPORTS1.1-master/source'
>> ~/.bashrc
```

chmod 755 your_path_to_SPORTS1.1-master/source/sports.pl

- 2. Install Bowtie
 - 1. Unpack bowtie-1.x.x-linux-x86 64.zip.

```
unzip bowtie-1.x.x-linux-x86_64.zip
```

2. Attach the bowtie directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_bowtie' >>
~/.bashrc
```

If you are administrator user, type the following command and password to easily in

```
sudo apt-get install bowtie
```

- 3. Install SRA Toolkit
 - 1. Unpack SRA toolkit files.
 - 2. Attach the SRA Toolkit executable path to your PATH:

```
echo 'export PATH=$PATH:your_path_to_sra-toolkit/bin'
>> ~/.bashrc
```

- 4. Install cutadapt
 - 1. Use pip on the command line to install latest version of cutadapt:

```
pip install --user --upgrade cutadapt
```

```
2. Attach the cutadapt directory to your PATH:
           echo 'export PATH=$PATH:$HOME/.local/bin' >> ~/.bashrc
    5. Install R and R package
        1. Unpack R-x.y.z.tar.gz with:
           tar -xf R-x.y.z.tar.gz
        2. Enter into the R-x.y.z directory:
           cd R-x.y.z
        3. Type following command in terminal:
           ./configure
           make
           make check
           make install
        4. Install R packages by typing following command in terminal:
           R
           install.packages('ggplot2', dependencies=TRUE, repos='http://cran.rstudio.com/'
           install.packages('data.table', dependencies=TRUE, repos='http://cran.rstudio.co
           install.packages('stringr', dependencies=TRUE, repos='http://cran.rstudio.com/'
           q()
4. Start a new shell session to apply changes to environment variables:
  source ~/.bashrc
5. Test if everything is installed properly:
  perl -v
  sports.pl -h
  bowtie
  fastq-dump
```

cutadapt -h

```
R --version
```

If you get any error messages you should install the software once again.

Script description

sports.pl

```
1. Input query format:
    1. .sra files.
    2. .fastq/.fq, .fasta/.fa files of deep sequencing reads.
  Attention: compressed files need to be unpacked before input!
2. Options:
  -Input:
  -i <file> Input could be:
      a .sra, .fastq/.fq or .fasta/.fa file;
      a directory (will run all qualified files in the directory recursively);
      a text document with absolute path information for each file/folder (when processing
  -Output:
  -o <str> output address of annotation results (default: input address)
  -k keep all the intermediate files generated during the running progress
  -Alignment:
  -1 <int> the minimal length of the output sequences (default = 15)
  -L <int> the maximal length of the output sequences (default = 45)
  -M <int> the total number of mismatches in the entire alignment (default = 0)
  -a Remove 5' / 3' adapters
      -x <str> (if -a applied) 5' adapter sequence. Default = "GTTCAGAGTTCTACAGTCCGACGATC
      -y <str> (if -a applied) 3' adapter sequence. Default = "TGGAATTCTCGGGTGCCAAGG"
  -Others:
```

-v print version information

-h print this usage message

3. Example

• Example use 1:

The user wants to map a single fasta file against rat reference genome to get the mapping genome annotation only. (No output figures)

Type following command in terminal:

sports.pl -i reads.fa -g /foo/bar/Rattus_norvegicus/UCSC/rn6/Sequence/BowtieIndex/genome

• Example use 2:

The user wants to map several already trimed human sequencing files to human reference genome, miRNA database, tRNA database, rRNA database and piRNA database by using 4 CPU threads, then to output the result to the address: '/foo/bar/output/'.

Write all the fastq files' addresses into a text document, e.g.:

```
seq_address.txt
```

/foo/bar/fold_1/seq_1.fastq /foo/bar/fold_2/seq_2.fq /foo/bar/fold_2/seq_3.fq /foo/bar/fold_3/seq_4.fasta /foo/bar/fold_4/seq_5.fa

Type following command in terminal:

sports.pl -i seq_address.txt -p 4 -g /foo/bar/Homo_sapiens/genome/hg38/genome -m /Homo_sapiens/miRBase/21/miRBase_21-has -r /foo/bar/Homo_sapiens/rRNAdb/human_rRNA -t /foo/bar/Homo_sapiens/GtRNAdb/hg19/hg19-tRNAs -w /foo/bar/Homo_sapiens/piRBase/piR_b-o /foo/bar/output/

• Example use 3:

The user wants to map several untrimmed mouse sequencing files downloaded from NCBI or somewhere else to mouse reference genome, miRNA database, tRNA database, rRNA database, piRNA database, ensembl noncoding RNA database and Rfam database by using 4 CPU threads, then to output the result to the address: '/foo/bar/output/' and keep all the intermediate files generated during the running progress.

Put all the sequencing files into a folder, e.g.:

folder structure:

```
download_seq
      fold_1
          seq_1.sra
          seq_2.sra
      fold_2
          fold_3
              seq_3.fastq
              seq_4.fq
          seq_5.fasta
      {\tt seq\_6.fa}
  Type following command in terminal:
  sports.pl -i /foo/bar/download_seq/ -p 4 -a -x GTTCAGAGTTCTACAGTCCGACGATC
  -y TGGAATTCTCGGGTGCCAAGG -g /foo/bar/Mus_musculus/genome/mm10/genome
  -m /foo/bar/Mus_musculus/miRBase/21/miRbase_21-mmu -r /foo/bar/Mus_musculus/rRNAdb/mous
  -t /foo/bar/Mus_musculus/GtRNAdb/mm10/mm10-tRNAs -w /foo/bar/Mus_musculus/piRBase/piR_m
  -e /foo/bar/Mus_musculus/Ensembl/Mus_musculus.GRCm38.ncrna
  -f /foo/bar/Mus_musculus/Rfam/12.3/Rfam-12.3-mouse -o /foo/bar/output/
4. Example output file structure for 1 query file input (e.g. SeqFile):
 Output folder structure
      1_SeqFile
         SeqFile_fa (if -k applied)
            SeqFile.fa
                                         ---unique seqs with reads number
            SeqFile_disgarded_reads.fa
                                                  ---seqs that cannot pass adapter removing
            SeqFile_too_short_reads.fa
                                                  ---seqs that are lower than required mini
            SeqFile_too_long_reads.fa
                                                  ---seqs that are higher than required max
```

```
SeqFile_match_genome.fa
                                         ---seqs that can match to reference genom
      SeqFile_unmatch_genome.fa
                                         ---seqs that cannot match to reference ge
      SeqFile_match_<X>_match_genome.fa
                                             ---seqs that can match to both <X> da
      SeqFile_match_<X>_unmatch_genome.fa
                                             ---seqs that can match to <X> databas
      SeqFile_unmatch_<X>_match_genome.fa
                                             ---segs that cannot match to <X> data
      SeqFile_unmatch_<X>_unmatch_genome.fa
                                                 ---seqs that match to <X> rfam da
  SeqFile_processed (if -k applied)
                                        ---seqs that match to reference genome in
      SeqFile_output_match_genome
      SeqFile_output_<X>_match_genome
                                             ---seqs that match to both miRNA data
      SeqFile_output_<X>_unmatch_genome
                                             ---seqs that match to miRNA database
  SeqFile_result
      SeqFile_output.txt
                                      ---6 column table file including annotation
                                      ---3 column table file including reads number
      SeqFile_summary.txt
      SeqFile_length_distribution.txt
                                              ---3 column table file including read
                                              ---11 column table file including mis
      SeqFile_mismatch_summary.txt
      SeqFile_sncRNA_distribution.pdf
                                             ---figure of length distribution of a
      SeqFile_rRNA_distribution.pdf
                                              ---figure of length distribution of
      SeqFile_rRNA_mapping.pdf
                                          ---figure of rsRNAs mapping against diffe
      SeqFile_tRNA_mapping.pdf
                                          ---figure of length distribution of diffe
processing_report (if -k applied)
   1_SeqFile.txt
                                  ---processing log file
sh_file (if -k applied)
```

---processing script file

1__SeqFile.sh

Some output folders only exist when '-k' parameter is applied in sports.pl;

SeqFile_mismatch_summary.txt only exist when '-M' parameter is lager than 0 in sports.pl

Some output files might not exist if the file size is zero.

fastq2fasta.pl

Extracted from miRDeep2 [3] (https://github.com/rajewsky-lab/mirdeep2)

1. Description:

Parses fastq format files into fasta format.

2. Input:

A fastq file.

3. Output:

A fasta file, one sequence per line (the sequences are expanded).

4. options:

_

5. Example usage:

fastq2fasta.pl reads.fq > reads.fa

fastaparse.pl

Extracted from miRDeep2 [3] (https://github.com/rajewsky-lab/mirdeep2)

1. Description:

Performs simple filtering of entries in a fasta file.

2. Input:

A fasta file

3. Output:

A filtered fasta file

4. Options:

- -a only output entries where the sequence is minimum int nts long
- -b remove all entries that have a sequence that contains letters other than a,c,g,t,u,n,A,C,G,T,U,N.
- -s output progress

5. Example usage:

```
fastaparse.pl reads.fa -a 15 -s > reads_no_short.fa 2>
reads_discarded.fa
```

${\bf combine_reads.pl}$

1. Description:

Combine reads in the fasta file to get unique sequence and its read number.

2. Input:

A fasta file

3. Output:

```
A filtered fasta file.
```

>t00000001 1234567

TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGC

't00000001' is the unique ID of the sequence, representing the abundance ranking among a '1234567' represents the reads number of sequence 'TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGC'

4. Options:

_

5. Example usage:

combine_reads.pl reads.fa > combined_reads.fa

tRNA_db_processing.pl

1. Description:

This script adds CCA end to original genomic tRNA 3' end and add G to Histidine tRNA 5' end.

2. Input:

The reference tRNA database file in .fa format

A fasta file

- 3. Options: -
- 4. Example usage:

tRNA_db_processing.pl input_tRNA_file.fa

annotation.pl

1. Description:

Combine the annotation information generated from sports.pl

2. Input:

sports.pl output folder address:

- 3. Output:
 - $1. \ \ \, \underline{\quad} \text{output.txt: A 6 column table file including annotation information} \\ \ \ \, \text{for every unique sequence.}$

	Sequence TCCCTGGTGGTCTAGTGGT	TAGGATTCGGCGC			Match_Genome Yes
-ID: t00000001			Represents the unique ID of the se		
-Sequence: TCCCTGGTGGTCTAGTGGTTAGGATTCG		.GGATTCGGCGC	Represents the sequence.		
-Length: 32			Length of the sequence.		
-Reads: 1234567			Reads number of the sequence.		
-Match_Genome: Yes			If the sequence can match the refe		
-Annotation: tRNA-Glu-CTC_5_end			The annotation of the sequence. The		
-	_summary.txt: A 3 colu ch major- and sub- classe		cluding read	ls	
number of ea	ich major- and sub- classe				
Class		Sub_Class - tRNA-Glu-CTC		Reads 123456	
Class tRNAdb-tRNA	5_end_Match_Genome [Adb-tRNA_5_end_Match_ tRNA-Glu-CTC_5_end	Sub_Class - tRNA-Glu-CTC GenomeTh	25_end e major cl e sub clas	Reads 123456 78910 ass name.	
Class tRNAdb-tRNAClass: tRN -Sub_Class: -Reads: 123 <seq_fold>_ ing reads num</seq_fold>	5_end_Match_Genome [Adb-tRNA_5_end_Match_ tRNA-Glu-CTC_5_end	Sub_Class - tRNA-Glu-CTC GenomeThTh A 3 column tab	e major cl e sub clas e reads nu ble file includajor class.	Reads 123456 78910 ass name. s name. mber of the	class.

```
tRNAdb-tRNA_5_end_Match_Genome 30 1234
tRNAdb-tRNA_5_end_Match_Genome 31 23456
tRNAdb-tRNA_5_end_Match_Genome 32 34567
tRNAdb-tRNA_5_end_Match_Genome 33 4567
```

.

._____

```
-Class: tRNAdb-tRNA_5_end_Match_Genome --The major class name.
-Length: 30 --Length of the sequence.
```

-Reads: 1234 -- The reads number of the class.

4. Options:

_

5. Example usage:

```
annotation.pl <SPORTS_output_fold_address>
```

tRNA_mapping.pl

1. Description:

Perl script calculates expression level of genomic loci by bowtie 1 output information.

2. Input:

bowtie 1 output file

3. Output:

expression summary file

4. Options:

-

5. Example usage:

```
tRNA_mapping.pl bowtie_output_file summary_file
```

mismatch_summary.pl

1. Description:

Perl script summarizes mismatch information generated by Bowtie 1 if parameter '-M' >0 applied in sports.pl

2. Input:

bowtie 1 output file and threshold of reads number.

3. Output:

mismatch summary file

4. Options:

_

5. Example usage:

mismatch_summary.pl bowtie_output_file 10 > mismatch_summary.txt

$overall_RNA_length_distribution.R$

1. Description:

Generate figure of length distribution of miRNA, rsRNA, tsRNA, piRNA and other RNAs, if sequence matches exists.

2. Input:

Files generated by annotation.pl

3. Output:

<seq_fold>_sncRNA_distribution.pdf Generate figure of length distribution of different types of rRNAs, if sequence matches exists.

4. Options:

_

5. Example usage:

Rscript --vanilla overall_RNA_length_distribution.R <SPORTS_output_fold_address>
<dataset_name>

rRNA_length_distribution.R

1. Description:

Generate figure of length distribution of different types of rRNAs, if sequence matches exists. (e.g. 4.5S, 5S, 5.3S, 5.8S, 12S, 16S, 18S, 28S, 45S ...)

2. Input:

Files generated by annotation.pl

3. Output:

<seq_fold>_rRNA_distribution.pdf

4. Options:

_

5. Example usage:

Rscript --vanilla r_RNA_length_distribution.R <SPORTS_output_fold_address>
<dataset_name>

rRNA_mapping.R

1. Description:

Generate figure of rsRNAs mapping against different types of rRNAs if sequence matches existed. (e.g. 4.5S, 5S, 5.3S, 5.8S, 12S, 16S, 18S, 28S, 45S ...)

2. Input:

Files generated by annotation.pl

3. Output:

```
<seq_fold>_rRNA_mapping.pdf
```

4. Options:

_

5. Example usage:

Rscript --vanilla rRNA_mapping.R <SPORTS_output_fold_address>
<dataset_name>

tRNA_mapping.R

1. Description:

Generate figure of rsRNAs mapping against different types of tRNAs if sequence matches existed.

2. Input:

Files generated by tRNA_mapping.pl

3. Output:

```
<seq_fold>_tRNA_mapping.pdf
```

4. Options:

_

5. Example usage:

```
Rscript --vanilla tRNA_mapping.R <SPORTS_output_fold_address>_tRNA_mapping.txt <dataset_name>_tRNA_mapping.pdf
```

mismatch stat.R

1. Description:

Generate statistics result for mismatch information generated from mismatch_summary.pl

2. Input:

File generated by mismatch_summary.pl and base-calling error

3. Output:

```
<seq_fold>_mismatch_summary.txt
```

4. Options:

_

5. Example usage:

```
Rscript --vanilla mismatch_stat.R <SPORTS_output_fold_address>_mismatch_summary.txt 0.01
```

Pre-compiled annotation databases instruction

Available species lists of bowtie-index based reference database

To build bowtie-index of your own:

```
cd /foo/bar/your_reference_database/
    bowtie-build <your_reference_database_name>.fa <your_reference_database_name>
The built bowtie index will be: /foo/bar/your_reference_database/your_reference_database
Unpack reference genome (e.g. human genome):
    tar zxvf Homo_sapiens_UCSC_hg38.tar.gz
Main database source:
-mirbase database [4] (Original source: http://www.mirbase.org/index.shtml)
-rRNA database (Original source: https://www.ncbi.nlm.nih.gov/nuccore)
-GtRNAdb database [5] (Original source: http://gtrnadb.ucsc.edu/)
-mitotRNAdb database [6] (Original source: http://mttrna.bioinf.uni-leipzig.de/mtDataOutput/
```

- -piRBase database [7] (Original source: http://www.regulatoryrna.org/database/piRNA/)
- -piRNABank [8] (Original source: http://pirnabank.ibab.ac.in/index.shtml)
- -ensembl ncRNA database [9] (Original source: http://www.ensembl.org/index.html)
- -rfam database [10] (Original source: http://rfam.xfam.org/)

To download annotation databases of mulitple species please use this link: $https://ncrnainfo-my.sharepoint.com/:f:/g/personal/sports_ncrna_info/EvZP50IfGFRJlNJwZRujI00BM-CP_w7gITsv8iU45m1rxQ?e=Pk89b9$

- 1. Homo sapiens (Human)
 - 1. annotation databases: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest 8CDc)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Homo_sapiens/genome/hg38/genome
 - -m /<your_defined_address>/Homo_sapiens/miRBase/21/miRBase_21-hsa
 - -r /<your_defined_address>/Homo_sapiens/rRNAdb/human_rRNA
 - -t /<your_defined_address>/Homo_sapiens/GtRNAdb/hg19/hg19-tRNAs
 - -w /<your_defined_address>/Homo_sapiens/piRBase/piR_human
 - -e /<your_defined_address>/Homo_sapiens/Ensembl/release-89/Homo_sapiens.GRCh38.ncrr
 - -f /<your_defined_address>/Homo_sapiens/Rfam/12.3/Rfam-12.3-human
- 2. Gorilla gorilla (Gorilla)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest IjvagRWePhYNF3k)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Gorilla_gorilla/UCSC/gorGor5/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Gorilla_gorilla/miRBase_21/miRBase_21-ggo
 - -t /<your_defined_address>/Gorilla_gorilla/GtRNAdb/gorGor3-tRNAs
 - -f /<your_defined_address>/Gorilla_gorilla/Rfam_12.3/Rfam-12.3-gorilla
- 3. Pan paniscus (Bonobo)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:

- -g /<your_defined_address>/Pan_paniscus/UCSC/panPan1/Sequence/BowtieIndex/genome
- -m /<your_defined_address>/Pan_paniscus/miRBase_21/miRBase_21-ppa
- -f /<your_defined_address>/Pan_paniscus/Rfam_12.3/Rfam-12.3-Bonobo
- 4. Pan troglodytes (Chimp)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest SbiP2C6Qjpc)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Pan_troglodytes/UCSC/panTro4/Sequence/BowtieIndex/genome
 - $\verb|-m /<your_defined_address>/Pan_troglodytes/miRBase_21/miRBase_21-ptr|$
 - -t /<your_defined_address>/Pan_troglodytes/GtRNAdb/panTro4-tRNAs
 - -e /<your_defined_address>/Pan_troglodytes/Ensembl/Pan_troglodytes.CHIMP2.1.4.ncma
 - -f /<your_defined_address>/Pan_troglodytes/Rfam_12.3/Rfam-12.3-chimp
- 5. Pongo abelii (Orangutan)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Pongo_abelii/UCSC/ponAbe2/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Pongo_abelii/miRBase_21/miRBase_21-ppy
 - -t /<your_defined_address>/Pongo_abelii/GtRNAdb/ponAbe2-tRNAs
 - -e /<your_defined_address>/Pongo_abelii/Ensembl/Pongo_abelii.PPYG2.ncrna
 - -f /<your_defined_address>/Pongo_abelii/Rfam_12.3/Rfam-12.3-orangutan
- 6. Nomascus leucogenys (Gibbon)
 - $6.1.\ annotation\ database:\ (We\ provide\ a\ download\ link\ for\ all\ databases\ listed\ below:\ https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestacces listed listed$
 - 6.2 SPORTS1.1 related parameters if you download recommended reference database:

. . .

- -g /<your_defined_address>/Nomascus_leucogenys/UCSC/nomLeu3/Sequence/BowtieIndex/genome
- -t /<your_defined_address>/Nomascus_leucogenys/GtRNAdb/nomLeu3-tRNAs
- -f /<your_defined_address>/Nomascus_leucogenys/Rfam_12.3/Rfam-12.3-gibbon
- 7. Macaca mulatta (Rhesus)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest CESs)

- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Macaca_mulatta/UCSC/rheMac8/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Macaca_mulatta/miRBase_21/miRBase_21-mml
 - -r /<your_defined_address>/Macaca_mulatta/rRNAdb/rhesus_rRNA
 - -t /<your_defined_address>/Macaca_mulatta/GtRNAdb/rheMac3-tRNAs
 - -f /<your_defined_address>/Macaca_mulatta/Rfam_12.3/Rfam-12.3-rhesus
- 8. Papio anubis (Baboon)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest 9BLoH_Vmw)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Papio_anubis/UCSC/papAnu2/Sequence/BowtieIndex/genome
 - -t /<your_defined_address>/Papio_anubis/GtRNAdb/papAnu2-tRNAs
 - -f /<your_defined_address>/Papio_anubis/Rfam_12.3/Rfam-12.3-baboon
- 9. Callithrix jacchus (Marmoset)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest CWgupu6IA)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Callithrix_jacchus/UCSC/calJac3/Sequence/BowtieIndex/gen
 - -m /<your_defined_address>/Callithrix_jacchus/rRNAdb/marmoset_rRNA
 - -t /<your_defined_address>/Callithrix_jacchus/GtRNAdb/calJac3-tRNAs
 - -f /<your_defined_address>/Callithrix_jacchus/Rfam_12.3/Rfam-12.3-marmoset
- 10. Carlito syrichta (Tarsier)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest hXdASOIimxNL4I)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Carlito_syrichta/UCSC/tarSyr2/Sequence/BowtieIndex/genor
 - -t /<your_defined_address>/Carlito_syrichta/GtRNAdb/tarSyr2-tRNAs
 - -f /<your_defined_address>/Carlito_syrichta/Rfam_12.3/Rfam-12.3-tarsier
- 11. Rattus norvegicus (rat)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest QMqBJoQ)
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Rattus_norvegicus/UCSC/rn6/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Rattus_norvegicus/miRBase_21/miRBase_21-rno
 - -r /<your_defined_address>/Rattus_norvegicus/rRNAdb/mouse_rRNA
 - -t /<your_defined_address>/Rattus_norvegicus/GtRNAdb/rn5-tRNAs
 - -w /<your_defined_address>/Rattus_norvegicus/piRBase/piR_rat
 - -e /<your_defined_address>/Rattus_norvegicus/Ensembl/Rattus_norvegicus.Rnor_6.0.ncm
 - -f /<your_defined_address>/Rattus_norvegicus/Rfam_12.3/Rfam-12.3-rat

12. Mus musculus (mouse)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Mus_musculus/genome/mm10/genome
 - -m /<your_defined_address>/Mus_musculus/miRBase/21/miRBase_21-mmu
 - -r /<your_defined_address>/Mus_musculus/rRNAdb/mouse_rRNA
 - -t /<your_defined_address>/Mus_musculus/GtRNAdb/mm10/mm10-tRNAs
 - -w /<your_defined_address>/Mus_musculus/piRBase/piR_mouse
 - -e /<your_defined_address>/Mus_musculus/Ensembl/release-89/Mus_musculus.GRCm38.ncm
 - -f /<your_defined_address>/Mus_musculus/Rfam/12.3/Rfam-12.3-mouse

13. Cricetulus griseus (Hamster)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Cricetulus_griseus/UCSC/criGri1/Sequence/BowtieIndex/gen
 - -m /<your_defined_address>/Cricetulus_griseus/miRBase_21/miRBase_21-cgr
 - -r /<your_defined_address>/Cricetulus_griseus/rRNA_db/hamster_rRNA
 - -t /<your_defined_address>/Cricetulus_griseus/GtRNAdb/criGri1-tRNAs
 - -f /<your_defined_address>/Cricetulus_griseus/Rfam_12.3/Rfam-12.3-hamster

14. Cavia porcellus (Guinea pig)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:

- -g /<your_defined_address>/Cavia_porcellus/UCSC/cavPor3/Sequence/BowtieIndex/genome
- -r /<your_defined_address>/Cavia_porcellus/rRNA_db/guinea_rRNA
- -t /<your_defined_address>/Cavia_porcellus/GtRNAdb/cavPor3-tRNAs
- -f /<your_defined_address>/Cavia_porcellus/Rfam_12.3/Rfam-12.3-guinea
- 15. Heterocephalus glaber (Naked mole-rat)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Heterocephalus_glaber/UCSC/hetGla2/Sequence/BowtieIndex,
 - -r /<your_defined_address>/Heterocephalus_glaber/rRNA_db/mole_rRNA
 - -t /<your_defined_address>/Heterocephalus_glaber/GtRNAdb/hetGla2-tRNAs
 - -f /<your_defined_address>/Heterocephalus_glaber/Rfam_12.3/Rfam-12.3-mole
- 16. Ictidomys tridecemlineatus (Squirrel)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Ictidomys_tridecemlineatus/UCSC/speTri2/Sequence/Bowtie
 - -t /<your_defined_address>/Ictidomys_tridecemlineatus/GtRNAdb/speTri2-tRNAs
 - -f /<your_defined_address>/Ictidomys_tridecemlineatus/Rfam_12.3/Rfam-12.3-squirrel
- 17. Ochotona princeps (Pika)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest DcRVQ)
 - SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Ochotona_princeps/UCSC/ochPri3/Sequence/BowtieIndex/genders
 - -t /<your_defined_address>/Ochotona_princeps/GtRNAdb/ochPri3-tRNAs
 - -f /<your_defined_address>/Ochotona_princeps/Rfam_12.3/Rfam-12.3-pika
- 18. Oryctolagus cuniculus (Rabbit)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest WJX10nDdXwvNygc)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Oryctolagus_cuniculus/UCSC/oryCun2/Sequence/BowtieIndex,
 - -m /<your_defined_address>/Oryctolagus_cuniculus/miRBase_21/miRBase_21-ocu

- -r /<your_defined_address>/Oryctolagus_cuniculus/rRNA_db/rabbit_rRNA
- -t /<your_defined_address>/Oryctolagus_cuniculus/GtRNAdb/oryCun2-tRNAs
- -f /<your_defined_address>/Oryctolagus_cuniculus/Rfam_12.3/Rfam-12.3-rabbit

19. Ovis aries (Sheep)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Ovis_aries/UCSC/oviAri3/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Ovis_aries/miRBase_21/miRBase_21-oar
 - -t /<your_defined_address>/Ovis_aries/GtRNAdb/oviAri1-tRNAs
 - -f /<your_defined_address>/Ovis_aries/Rfam_12.3/Rfam-12.3-sheep

20. Bos taurus (Cow)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Bos_taurus/UCSC/bosTau8/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Bos_taurus/miRBase_21/miRBase_21-bta
 - -r /<your_defined_address>/Bos_taurus/rRNA_db/cow_rRNA
 - -t /<your_defined_address>/Bos_taurus/GtRNAdb/bosTau8-tRNAs
 - -e /<your_defined_address>/Bos_taurus/Ensembl/Bos_taurus.UMD3.1.ncrna
 - -f /<your_defined_address>/Bos_taurus/Rfam_12.3/Rfam-12.3-cow

21. Sus scrofa (Pig)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Sus_scrofa/UCSC/susScr3/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Sus_scrofa/miRBase_21/miRBase_21-ssc
 - -r /<your_defined_address>/Sus_scrofa/rRNA_db/pig_rRNA
 - -t /<your_defined_address>/Sus_scrofa/GtRNAdb/susScr3-tRNAs
 - -f /<your_defined_address>/Sus_scrofa/Rfam_12.3/Rfam-12.3-pig

22. Tursiops truncatus (Dolphin)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:

- -g /<your_defined_address>/Tursiops_truncatus/UCSC/turTru2/Sequence/BowtieIndex/gen
- -t /<your_defined_address>/Tursiops_truncatus/GtRNAdb/turTru2-tRNAs
- -f /<your_defined_address>/Tursiops_truncatus/Rfam_12.3/Rfam-12.3-dolphin
- 23. Balaenoptera acutorostrata (Minke whale)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest_q0lzVEEBlth8V8)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - $\verb|-g|/<your_defined_address>/Balaenoptera_acutorostrata/UCSC/balAcu1/Sequence/Bowtie-Response for the state of the state$
 - -t /<your_defined_address>/Balaenoptera_acutorostrata/GtRNAdb/balAcu1-tRNAs
 - -f /<your_defined_address>/Balaenoptera_acutorostrata/Rfam_12.3/Rfam-12.3-whale
- 24. Erinaceus europaeus (Hedgehog)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Erinaceus_europaeus/UCSC/eriEur2/Sequence/BowtieIndex/ge
 - -t /<your_defined_address>/Erinaceus_europaeus/GtRNAdb/eriEur2-tRNAs
 - -f /<your_defined_address>/Erinaceus_europaeus/Rfam_12.3/Rfam-12.3-hedgehog
- 25. Sorex araneus (Shrew)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Sorex_araneus/UCSC/sorAra2/Sequence/BowtieIndex/genome
 - -t /<your defined address>/Sorex araneus/GtRNAdb/sorAra2-tRNAs
 - -f /<your_defined_address>/Sorex_araneus/Rfam_12.3/Rfam-12.3-shrew
- 26. Canis familiaris (Dog)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest C5u9DG71tzrFEI)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - $\verb|-g|/<your_defined_address>/Canis_familiaris/UCSC/canFam3/Sequence/BowtieIndex/genored by the state of the$
 - -m /<your_defined_address>/Canis_familiaris/miRBase_21/miRBase_21-cfa
 - -t /<your_defined_address>/Canis_familiaris/GtRNAdb/canFam3-tRNAs
 - -f /<your_defined_address>/Canis_familiaris/Rfam_12.3/Rfam-12.3-dog

- 27. Mustela putorius furo (Ferret)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Mustela_furo/UCSC/musFur1/Sequence/BowtieIndex/genome
 - -t /<your_defined_address>/Mustela_furo/GtRNAdb/musFur1-tRNAs
 - -f /<your_defined_address>/Mustela_furo/Rfam_12.3-ferret
- 28. Ailuropoda melanoleuca (Panda)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Ailuropoda_melanoleuca/UCSC/ailMel1/Sequence/BowtieIndex
 - -t /<your_defined_address>/Ailuropoda_melanoleuca/GtRNAdb/ailMel1-tRNAs
 - -f /<your_defined_address>/Ailuropoda_melanoleuca/Rfam-12.3-panda
- 29. Felis catus (Cat)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Felis_catus/UCSC/felCat8/Sequence/BowtieIndex/genome
 - -r /<your_defined_address>/Felis_catus/rRNA_db/cat_rRNA
 - -t /<your_defined_address>/Felis_catus/GtRNAdb/felCat5-tRNAs
 - -f /<your_defined_address>/Felis_catus/Rfam_12.3/Rfam-12.3-cat
- 30. Equus caballus (Horse)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Equus_caballus/UCSC/equCab2/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Equus_caballus/miRBase_21/miRBase_21-eca
 - -r /<your_defined_address>/Equus_caballus/rRNA_db/horse_rRNA
 - -t /<your_defined_address>/Equus_caballus/GtRNAdb/equCab2-tRNAs
 - -f /<your_defined_address>/Equus_caballus/Rfam_12.3/Rfam-12.3-horse
- 31. Ceratotherium simum (White rhinoceros)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - $\verb|-g|/span=2 fined_address|/ceratotherium_simum/UCSC/cerSim1/Sequence/BowtieIndex/general fined_address|/span=2 fined_address|/spa$
 - -t /<your_defined_address>/Ceratotherium_simum/GtRNAdb/cerSim1-tRNAs
 - -f /<your_defined_address>/Ceratotherium_simum/Rfam_12.3/Rfam-12.3-rhinoceros

32. Myotis lucifugus (Microbat)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest 2sRUrTGn7k)
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Myotis_lucifugus/UCSC/myoLuc2/Sequence/BowtieIndex/genor
 - -t /<your_defined_address>/Myotis_lucifugus/GtRNAdb/myoLuc2-tRNAs
 - -f /<your_defined_address>/Myotis_lucifugus/Rfam_12.3/Rfam-12.3-bat
- 33. Trichechus manatus (Manatee)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - $\verb|-g|/<your_defined_address>/Trichechus_manatus/UCSC/triMan1/Sequence/BowtieIndex/general-control of the control of the cont$
 - -t /<your_defined_address>/Trichechus_manatus/GtRNAdb/triMan1-tRNAs
 - -f /<your_defined_address>/Trichechus_manatus/Rfam_12.3/Rfam-12.3-manatee
- 34. Loxodonta africana (Elephant)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest 7s)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Loxodonta_africana/UCSC/loxAfr3/Sequence/BowtieIndex/gen
 - -t /<your_defined_address>/Loxodonta_africana/GtRNAdb/loxAfr3-tRNAs
 - -f /<your_defined_address>/Loxodonta_africana/Rfam_12.3/Rfam-12.3-elephant
- 35. Dasypus novemcinctus (Armadillo)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest

- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Dasypus_novemcinctus/UCSC/dasNov3/Sequence/BowtieIndex/g
 - -t /<your_defined_address>/Dasypus_novemcinctus/GtRNAdb/dasNov3-tRNAs
 - -f /<your_defined_address>/Dasypus_novemcinctus/Rfam_12.3/Rfam-12.3-armadillo
- 36. Notamacropus eugenii (Wallaby)
 - annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest dFi1Mm7C0QH9K00WHH4)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Notamacropus_eugenii/UCSC/macEug2/Sequence/BowtieIndex/g
 - -m /<your_defined_address>/Notamacropus_eugenii/miRBase_21/miRBase_21-meu
 - -t /<your_defined_address>/Notamacropus_eugenii/GtRNAdb/macEug2-tRNAs
 - -f /<your_defined_address>/Notamacropus_eugenii/Rfam_12.3/Rfam-12.3-wallaby
- 37. Sarcophilus harrisii (Tasmanian devil)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest D6mL0)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Sarcophilus_harrisii/UCSC/sarHar1/Sequence/BowtieIndex/8
 - -m /<your_defined_address>/Sarcophilus_harrisii/miRBase_21/miRBase_21-sha
 - -t /<your_defined_address>/Sarcophilus_harrisii/GtRNAdb/sarHar1-tRNAs
 - -f /<your_defined_address>/Sarcophilus_harrisii/Rfam_12.3/Rfam-12.3-tasmanian
- 38. Monodelphis domestica (Opossum)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Monodelphis_domestica/UCSC/monDom5/Sequence/BowtieIndex,
 - -m /<your_defined_address>/Monodelphis_domestica/miRBase_21/miRBase_21-mdo
 - -t /<your_defined_address>/Monodelphis_domestica/GtRNAdb/monDom5-tRNAs
 - -e /<your_defined_address>/Monodelphis_domestica/Ensembl/Monodelphis_domestica.BROA
 - -f /<your_defined_address>/Monodelphis_domestica/Rfam_12.3/Rfam-12.3-opossum
- 39. Ornithorhynchus anatinus (Platypus)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest wfpJ81M)
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Ornithorhynchus_anatinus/UCSC/ornAna2/Sequence/BowtieInd
 - -m /<your_defined_address>/Ornithorhynchus_anatinus/miRBase_21/miRBase_21-oan
 - -t /<your_defined_address>/Ornithorhynchus_anatinus/GtRNAdb/ornAna1-tRNAs
 - -w /<your_defined_address>/Ornithorhynchus_anatinus/piRBase/piR_platypus
 - -e /<your_defined_address>/Ornithorhynchus_anatinus/Ensembl/Ornithorhynchus_anatinus
 - -f /<your_defined_address>/Ornithorhynchus_anatinus/Rfam_12.3/Rfam-12.3-platypus
- 40. Taeniopygia guttata (Zebra finch)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Taeniopygia_guttata/UCSC/taeGut2/Sequence/BowtieIndex/ge
 - -m /<your_defined_address>/Taeniopygia_guttata/miRBase_21/miRBase_21-tgu
 - -t /<your_defined_address>/Taeniopygia_guttata/GtRNAdb/taeGut2-tRNAs
 - -f /<your_defined_address>/Taeniopygia_guttata/Rfam_12.3/Rfam-12.3-finch
- 41. Melopsittacus undulatus (Budgerigar)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Melopsittacus_undulatus/UCSC/melUnd1/Sequence/BowtieInde
 - -t /<your_defined_address>/Melopsittacus_undulatus/GtRNAdb/melUnd1-tRNAs
 - -f /<your_defined_address>/Melopsittacus_undulatus/Rfam_12.3/Rfam-12.3-budgerigar
- 42. Gallus gallus (Chicken)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. PORTS1.0 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Gallus_gallus/UCSC/galGal5/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Gallus_gallus/miRBase_21/miRBase_21-gga
 - -r /<your_defined_address>/Gallus_gallus/rRNA_db/chicken_rRNA
 - -t /<your_defined_address>/Gallus_gallus/GtRNAdb/galGal4-tRNAs
 - -w /<your_defined_address>/Gallus_gallus/piRBase/piR_gga_v1.0
 - -e /<your_defined_address>/Gallus_gallus/Ensembl/Gallus_gallus.Gallus_gallus-5.0.nd

- -f /<your_defined_address>/Gallus_gallus/Rfam_12.3/Rfam-12.3-chicken
- 43. Meleagris gallopavo (Turkey)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - $\verb|-g|/<your_defined_address>/Meleagris_gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGal1/Sequence/BowtieIndex/general-gallopavo/UCSC/melGallopavo/UCS$
 - -t /<your_defined_address>/Meleagris_gallopavo/GtRNAdb/melGal1-tRNAs
 - -f /<your_defined_address>/Meleagris_gallopavo/Rfam_12.3/Rfam-12.3-turkey
- 44. Chrysemys picta (Painted Turtle)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Chrysemys_picta/UCSC/chrPic1/Sequence/BowtieIndex/genome
 - -t /<your_defined_address>/Chrysemys_picta/GtRNAdb/chrPic1-tRNAs
 - -f /<your_defined_address>/Chrysemys_picta/Rfam_12.3/Rfam-12.3-turtle
- 45. Anolis carolinensis (Lizard)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest i58-1fjJeqcDHZUpLLo)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Anolis_carolinensis/UCSC/anoCar2/Sequence/BowtieIndex/ge
 - -m /<your_defined_address>/Anolis_carolinensis/miRBase_21/miRBase_21-aca
 - -t /<your_defined_address>/Anolis_carolinensis/GtRNAdb/anoCar2-tRNAs
 - -e /<your_defined_address>/Anolis_carolinensis/Ensembl/Anolis_carolinensis.AnoCar2
 - -f /<your_defined_address>/Anolis_carolinensis/Rfam_12.3/Rfam-12.3-lizard
- 46. Xenopus laevis (Frog)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Xenopus_laevis/UCSC/xenTro7/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Xenopus_laevis/miRBase_21/miRBase_21-xtr
 - -r /<your_defined_address>/Xenopus_laevis/rRNA_db/frog_rRNA
 - -t /<your_defined_address>/Xenopus_laevis/GtRNAdb/xenTro3-tRNAs
 - -w /<your_defined_address>/Xenopus_laevis/piRBase/piR_xtr_v1.0

- -e /<your_defined_address>/Xenopus_laevis/Ensembl/Xenopus_tropicalis.JGI_4.2.ncrna
- -f /<your_defined_address>/Xenopus_laevis/Rfam_12.3/Rfam-12.3-frog

47. Latimeria chalumnae (Coelacanth)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Latimeria_chalumnae/UCSC/latCha1/Sequence/BowtieIndex/ge
 - -r /<your_defined_address>/Latimeria_chalumnae/rRNA_db/coelacanth_rRNA
 - -t /<your_defined_address>/Latimeria_chalumnae/GtRNAdb/latCha1-tRNAs
 - -f /<your_defined_address>/Latimeria_chalumnae/Rfam_12.3/Rfam-12.3-coelacanth

48. Tetraodon nigroviridis (Tetraodon)

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest iphiQFRDzuKoJtA)
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Tetraodon_nigroviridis/UCSC/tetNig2/Sequence/BowtieIndex
 - -m /<your_defined_address>/Tetraodon_nigroviridis/miRBase_21/miRBase_21-tni
 - -t /<your_defined_address>/Tetraodon_nigroviridis/GtRNAdb/tetNig2-tRNAs
 - -f /<your_defined_address>/Tetraodon_nigroviridis/Rfam_12.3/Rfam-12.3-tetraodon

49. Takifugu rubripes (Fugu)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest 1dy5wnCfdQ)
- SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Takifugu_rubripes/UCSC/fr3/Sequence/BowtieIndex/genome
 - -t /<your_defined_address>/Takifugu_rubripes/GtRNAdb/fr3-tRNAs
 - -f /<your_defined_address>/Takifugu_rubripes/Rfam_12.3/Rfam-12.3-fugu

50. Gasterosteus aculeatus (Stickleback)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Gasterosteus_aculeatus/UCSC/gasAcu1/Sequence/BowtieInder
 - -t /<your_defined_address>/Gasterosteus_aculeatus/GtRNAdb/gasAcu1-tRNAs
 - -e /<your_defined_address>/Gasterosteus_aculeatus/Ensembl/Gasterosteus_aculeatus.BI

- -f /<your_defined_address>/Gasterosteus_aculeatus/Rfam_12.3/Rfam-12.3-stickleback
- 51. Oryzias latipes (Medaka)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Oryzias_latipes/UCSC/oryLat2/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Oryzias_latipes/miRBase_21/miRBase_21-ola
 - -t /<your_defined_address>/Oryzias_latipes/GtRNAdb/oryLat2-tRNAs
 - -f /<your_defined_address>/Oryzias_latipes/Rfam_12.3/Rfam-12.3-medaka
- 52. Oreochromis niloticus (Nile tilapia)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Oreochromis_niloticus/UCSC/oreNil2/Sequence/BowtieIndex
 - -t /<your_defined_address>/Oreochromis_niloticus/GtRNAdb/oreNil2-tRNAs
 - -f /<your_defined_address>/Oreochromis_niloticus/Rfam_12.3/Rfam-12.3-tilapia
- 53. Gadus morhua (Atlantic cod)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Gadus_morhua/UCSC/gadMor1/Sequence/BowtieIndex/genome
 - -r /<your defined address>/Gadus morhua/rRNA db/cod rRNA
 - -t /<your_defined_address>/Gadus_morhua/GtRNAdb/gadMor1-tRNAs
 - -f /<your_defined_address>/Gadus_morhua/Rfam_12.3/Rfam-12.3-cod
- 54. Danio rerio (Zebrafish)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest VjshX-GKLZI7limHFIo)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Danio_rerio/UCSC/danRer10/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Danio_rerio/miRBase_21/miRBase_21-dre
 - -r /<your_defined_address>/Danio_rerio/rRNA_db/zebrafish_rRNA
 - -t /<your_defined_address>/Danio_rerio/GtRNAdb/danRer6-tRNAs
 - -w /<your_defined_address>/Danio_rerio/piRBase/piR_dre_v1.0

- -e /<your_defined_address>/Danio_rerio/Ensembl/Danio_rerio.GRCz10.ncrna
- -f /<your_defined_address>/Danio_rerio/Rfam_12.3/Rfam-12.3-zebrafish
- 55. Callorhinchus milii (Elephant shark)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Callorhinchus_milii/UCSC/calMil1/Sequence/BowtieIndex/ge
 - -t /<your_defined_address>/Callorhinchus_milii/GtRNAdb/calMil1-tRNAs
 - -f /<your_defined_address>/Callorhinchus_milii/Rfam_12.3/Rfam-12.3-shark
- 56. Petromyzon marinus (Lamprey)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Petromyzon_marinus/UCSC/petMar2/Sequence/BowtieIndex/gen
 - -r /<your_defined_address>/Petromyzon_marinus/rRNA_db/lamprey_rRNA
 - -t /<your_defined_address>/Petromyzon_marinus/GtRNAdb/petMar2-tRNAs
 - -f /<your_defined_address>/Petromyzon_marinus/Rfam_12.3/Rfam-12.3-lamprey
- 57. Strongylocentrotus purpuratus (Sea urchin)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Strongylocentrotus_purpuratus/UCSC/strPur2/Sequence/Bowl
 - -m /<your_defined_address>/Strongylocentrotus_purpuratus/miRBase_21/miRBase_21-spu
 - -r /<your_defined_address>/Strongylocentrotus_purpuratus/rRNA_db/urchin_rRNA
 - -t /<your_defined_address>/Strongylocentrotus_purpuratus/GtRNAdb/Spurp-tRNAs
 - -e /<your_defined_address>/Strongylocentrotus_purpuratus/Ensembl/Strongylocentrotus
 - -f /<your_defined_address>/Strongylocentrotus_purpuratus/Rfam_12.3/Rfam-12.3-urchin
- 58. Drosophila melanogaster (Drosophila)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Drosophila_melanogaster/UCSC/dm6/Sequence/BowtieIndex/ge
 - -m /<your_defined_address>/Drosophila_melanogaster/miRBase_21/miRBase_21-dme
 - -r /<your_defined_address>/Drosophila_melanogaster/rRNA_db/drosophila_rRNA

- -t /<your_defined_address>/Drosophila_melanogaster/GtRNAdb/dm6-tRNAs
- -w /<your_defined_address>/Drosophila_melanogaster/piRBase/piR_dme
- -e /<your_defined_address>/Drosophila_melanogaster/Ensembl/Drosophila_melanogaster
- -f /<your_defined_address>/Drosophila_melanogaster/Rfam_12.3/Rfam-12.3-drosophila
- 59. Anopheles gambiae (Mosquito)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Anopheles_gambiae/UCSC/anoGam1/Sequence/BowtieIndex/genders
 - -m /<your_defined_address>/Anopheles_gambiae/miRBase_21/miRBase_21-aga
 - -t /<your_defined_address>/Anopheles_gambiae/GtRNAdb/Agamb-tRNAs
 - $\hbox{-e /<your_defined_address>/Anopheles_gambiae/Ensembl/Anopheles_gambiae.AgamP4.ncmarked and address and address$
 - -f /<your_defined_address>/Anopheles_gambiae/Rfam_12.3/Rfam-12.3-mosquito
- 60. Pristionchus pacificus (Roundworm)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Pristionchus_pacificus/UCSC/priPac1/Sequence/BowtieIndex
 - $\verb|-m /<your_defined_address|| Pristionchus_pacificus/miRBase_21/miRBase_21-ppc||$
 - $\verb|-r|/<your_defined_address|| Pristionchus_pacificus/rRNA_db/roundworm_rRNA|| and the substitution of th$
 - -t /<your_defined_address>/Pristionchus_pacificus/GtRNAdb/priPac1-tRNAs
 - -f /<your_defined_address>/Pristionchus_pacificus/Rfam_12.3/Rfam-12.3-roundworm
- 61. Caenorhabditis elegans (Nematode):
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest pCI)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Caenorhabditis_elegans/UCSC/ce10/Sequence/BowtieIndex/ge
 - -m /<your_defined_address>/Caenorhabditis_elegans/miRBase_21/miRBase_21-cel
 - -r /<your_defined_address>/Caenorhabditis_elegans/rRNA_db/cel_rRNA
 - -t /<your_defined_address>/Caenorhabditis_elegans/GtRNAdb/ce10-tRNAs
 - -w /<your_defined_address>/Caenorhabditis_elegans/piRBase/piR_cel_v1.0
 - -e /<your_defined_address>/Caenorhabditis_elegans/Ensembl/Caenorhabditis_elegans.Wl
 - -f /<your_defined_address>/Caenorhabditis_elegans/Rfam_12.3/Rfam-12.3-nematode
- 62. Saccharomyces cerevisiae (Yeast)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Saccharomyces_cerevisiae/UCSC/sacCer3/Sequence/BowtieInd
 - -r /<your_defined_address>/Saccharomyces_cerevisiae/rRNA_db/yeast_rRNA
 - -t /<your_defined_address>/Saccharomyces_cerevisiae/GtRNAdb/sacCer3-tRNAs
 - -e /<your_defined_address>/Saccharomyces_cerevisiae/Ensembl/Saccharomyces_cerevisia
 - -f /<your_defined_address>/Saccharomyces_cerevisiae/Rfam_12.3/Rfam-12.3-yeast

63. Zea mays (Corn)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Zea_mays/Ensembl/AGPv4/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Zea_mays/miRBase_21/miRBase_21-zma
 - -r /<your_defined_address>/Zea_mays/rRNA_db/corn_rRNA
 - -t /<your_defined_address>/Zea_mays/GtRNAdb/zeaMay5-tRNAs
 - -e /<your_defined_address>/Zea_mays/Ensembl/Zea_mays.AGPv4.ncrna
 - -f /<your_defined_address>/Zea_mays/Rfam_12.3/Rfam-12.3-corn

64. Sorghum bicolor (Sorghum)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Sorghum_bicolor/Ensembl/Sbi1/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Sorghum_bicolor/miRBase_21/miRBase_21-sbi
 - -r /<your_defined_address>/Sorghum_bicolor/rRNA_db/sorghum_rRNA
 - -t /<your_defined_address>/Sorghum_bicolor/GtRNAdb/Sbico-tRNAs
 - -e /<your_defined_address>/Sorghum_bicolor/Ensembl/Sorghum_bicolor.Sorghum_bicolor
 - -f /<your_defined_address>/Sorghum_bicolor/Rfam_12.3/Rfam-12.3-sorghum

65. Oryza sativa (Rice)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
- 2. SPORTS1.1 related parameters if you download recommend reference database:
 - -g /<your_defined_address>/Oryza_sativa/Ensembl/IRGSP-1.0/Sequence/BowtieIndex/genders
 - -m /<your_defined_address>/Oryza_sativa/miRBase_21/miRBase_21-osa
 - -r /<your_defined_address>/Oryza_sativa/rRNA_db/rice_rRNA

- -t /<your_defined_address>/Oryza_sativa/GtRNAdb/Osati-tRNAs
- -e /<your_defined_address>/Oryza_sativa/Ensembl/Oryza_sativa.IRGSP-1.0.ncrna
- -f /<your_defined_address>/Oryza_sativa/Rfam_12.3/Rfam-12.3-rice
- 66. Arabidopsis thaliana (Arabidopsis)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Arabidopsis_thaliana/Ensembl/TAIR10/Sequence/BowtieIndex
 - -m /<your_defined_address>/Arabidopsis_thaliana/miRBase_21/miRBase_21-ath
 - -r /<your_defined_address>/Arabidopsis_thaliana/rRNA_db/Arabidopsis_rRNA
 - -t /<your defined address>/Arabidopsis thaliana/GtRNAdb/araTha1-tRNAs
 - -e /<your_defined_address>/Arabidopsis_thaliana/Ensembl/Arabidopsis_thaliana.TAIR10
 - -f /<your_defined_address>/Arabidopsis_thaliana/Rfam_12.3/Rfam-12.3-arabidopsis
- 67. Glycine max (Soybean)
 - 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Glycine_max/Ensembl/Gm01/Sequence/BowtieIndex/genome
 - -m /<your_defined_address>/Glycine_max/miRBase_21/miRBase_21-gma
 - -r /<your_defined_address>/Glycine_max/rRNA_db/soybean_rRNA
 - -t /<your_defined_address>/Glycine_max/GtRNAdb/glyMax2-tRNAs
 - -f /<your_defined_address>/Glycine_max/Rfam_12.3/Rfam-12.3-soybean
- 68. Escherichia coli (E.coli)
 - annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guest SpOIxaUik)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:
 - -g /<your_defined_address>/Escherichia_coli/Ensembl/EB1/Sequence/BowtieIndex/genome
 - -r /<your_defined_address>/Escherichia_coli/rRNA_db/e_coli_rRNA
 - -t /<your_defined_address>/Escherichia_coli/GtRNAdb/eschColi-tRNAs
 - -e /<your_defined_address>/Escherichia_coli/Ensembl/Escherichia_coli.HUSEC2011CHR1
 - -f /<your_defined_address>/Escherichia_coli/Rfam_12.3/Rfam-12.3-e_coli

Instruction for compiling annotation database by user

1. Prepare the reference databases for your species X

- 1. Download the reference genome in .fa format into the defined folder address: <your_defined_address>; (necessary)
- 2. Download and extract the miRNA sequences belong to the species in .fa format from miRBase (http://www.mirbase.org/), convert RNA sequences to cDNA sequences (U -> T), then put the file X_mirbase.fa into the defined folder address: <your_defined_address>; (optional)
- 3. Download the rRNA sequences in .fa format from NCBI or anywhere else, convert RNA sequences to cDNA sequences (U -> T), name and list each rRNA sequences separately, as exampled below, and put the files such X_rRNA_5S.fa, X_rRNA_5.8S.fa into the defined folder address: <your_defined_address>; (optional)

human rRNA 5S.fa

>Human 5S ribosomal RNA

 ${\tt GTCTACGGCCATACCACCCTGAACGCGCCCGATCTCGTCTGATCTCGGAAGCTAAGCAGGGTCGGGCCTGGTTAGTACTTGGATGGGAGACCGCCTGGGAATACCGGGTGCTGTAGGCTTT$

human rRNA 5.8S.fa

>Human 5.8S ribosomal RNA

 ${\tt CGACTCTTAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAACGCAGCGCTAGCTGCGAGAATTAATGTGAATTGCAGGACACATTGATCATCGACACTTCGAACGCACTTGCGGCCCCGGGGTTCCTCCCGGGGCTACGCCTGTCTGAGCGTCGCTT$

- 4. Download the high confidence tRNA sequences (X-tRNAs.fa) and high confidence mature tRNA sequences (X-mature-tRNAs.fa) in .fa format from GtRNAdb (http://gtrnadb.ucsc.edu/) and put the files X-mature-tRNAs.fa and X-tRNAs.fa into the defined folder address: <your_defined_address>; (optional)
- 5. Download the noncoding RNA sequences in .fa format from Ensembl database (http://ensemblgenomes.org/) and put the file X_ensembl_ncrna.fa into the defined folder address: <your defined address>; (optional)
- 6. Download and extract the noncoding RNA sequences in .fa format from Rfam database (http://ensemblgenomes.org/) and put the file X_rfam_ncrna.fa into the defined folder address: <your_defined_address>; (optional)
- 7. Download and extract the noncoding RNA sequences belong to the species in .fa format from Rfam database (https://rfam.xfam.org/) and put the file X_rfam.fa into the defined folder address: <your_defined_address>; (optional)
- 8. Download piRNA sequences in .fa format from piRNA databases and put the file X_piRNA.fa into the defined folder address:

```
<your_defined_address>. (optional)
```

2. Type following command in terminal:

```
sports.pl -i /foo/bar/download_seq/ -p 4 -g <your_defined_address>/X
-m <your_defined_address>/X_mirbase -r <your_defined_address>/X_rRNA
-t <your_defined_address>/X_tRNAs -e <your_defined_address>/X_ensembl_ncrna
-f <your_defined_address>/X_rfam_ncrna -w <your_defined_address>/X_piRNA
-o /foo/bar/output/
```

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Update history

- 1.1.0 02/20/2020 1. The tsRNA annotation information has been refined to pre-tsRNA and mature-tsRNA based on updated tRNA database; 2. Annotation for Mitocondrial tRNAs (obtained from mitotRNAdb http://mttrna.bioinf.uni-leipzig.de/mtDataOutput/) and yRNAs (obtained from https://www.ncbi.nlm.nih.gov/) are now available. Pre-compiled databases for mouse and human have been updated (databases for other species will updated soon if applicable), please re-download the databases for better results; 3. The miRNA annotation process has been optimized to get more accurate results. 4. Bowtie index building process has been imbedded in sports.pl to reduce the size of pre-compiled database; 5. New parameter '-z' is added to make mismatch information statistics optional ('-M' > 0 is needed for the statistics). 6. Instruction for compiling annotation database by user is added.
- 1.0.5 09/25/2018 1. Changed annotation strategy to fit more species tRNA annotation in GtRNAdb. 2. Fixed some bugs in the previous version. Thanks Catrin Lloyd to figure one out!
- 1.0.4 03/25/2018 1. New function added: if '-M' > 0 applied in sports.pl, statistics of mismatch information will generated to predict potential nucleic acid modification loci; 2. The figure of tRNA mapping information will generated if tRNA database is defined; 3. A sample output with the parameter '-M 1' of dataset GSM2304822 (mouse sperm) is uploaded here.
- 1.0.3 01/27/2018 1. Changed the mapping order to make the mapping results more accurate according to the paper "Juan Pablo Tosar, Carlos Rovira, Alfonso Cayota. Non-coding RNA fragments account for the majority of annotated piRNAs expressed in somatic non-gonadal tissues. Communications Biology. 2018;1, Article number: 2. doi: 10.1038/s42003-017-0001-7 ".
- 1.0.2 01/22/2018 1. Fixed bugs to generate more accurate mature tRNA database.
- 1.0.1 01/13/2018 1. Fixed bugs that caused unexpected exit. 2. Optimized script to speed up tRNA annotation process.

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