SPORTS1.1 (**S**mall non-coding RNA annotation **P**ipeline **O**ptimized for **r**RNA- and **t**RNA-Derived **S**mall RNAs)

If you use SPORTS1.1 in your work, please cite these papers:

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

Junchao Shi, Eun-A Ko, Kenton M. Sanders, Qi Chen, Tong Zhou. SPORTS1.0: a tool for annotating and profiling non-coding RNAs optimized for rRNA-and tRNA-derived small RNAs. *Genomics, Proteomics & Bioinformatics* (2018) doi.org/10.1016/j.gpb.2018.04.004

Optimized identification and characterization of small RNAs with PANDORA-seq

Junchao Shi, Yunfang Zhang, Yun Li, Liwen Zhang, Xudong Zhang, Menghong Yan, Qi Chen, Ying Zhang. Optimized identification and characterization of small RNAs with PANDORA-seq. *Nature Protocols* (2025) doi.org/10.1038/s41596-025-01158-4

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Instruction for compiling annotation database by user

Copyright and licensing information

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Disclaimer

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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://github.com/ncbi/sra-tools) (Tested version: 2.8.2) cutadapt [2] (http://cutadapt.readthedocs.io/en/stable/index.html) (Tested version: 1.11)
 - 4. R (https://www.r-project.org/) (Tested version: 3.2.3, 3.2.5)
 - 5. 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 install bowtie:
```

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:

```
install.packages('ggplot2', dependencies=TRUE,
repos='http://cran.rstudio.com/')
install.packages('data.table', dependencies=TRUE,
repos='http://cran.rstudio.com/')
install.packages('stringr', dependencies=TRUE,
repos='http://cran.rstudio.com/')
q()
n
```

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 suffix .txt) with absolute path information for each file/folder (when processing multiple data, input each file/folder path per line)
```

--Output:

```
-o <str> output address of annotation results (default: input address)
```

-k keep all the intermediate files generated during the running progress

--Alignment:

```
-l <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

Detailed instructions for running the demo small RNA-seq data analysis, along with example output results, are provided in the Demo folder.

• 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
```

o 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 with suffix .txt, e.g.:

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_human -o /foo/bar/output/
```

o 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

fold_3

seq_4.fq

seq_4.fq

seq_5.fasta
```

```
|
└─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/mouse_rRNA -t /foo/bar/Mus_musculus/GtRNAdb/mm10/mm10-
tRNAs -w /foo/bar/Mus_musculus/piRBase/piR_mouse -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/ -k
```

4. Example output file structure for 1 query file input (e.g. SeqFile):

```
Output folder structure
       —1 SegFile
          ├─SeqFile_fa (if -k applied)
              -SegFile.fa
                                       ---unique seqs with reads number
              ---seqs that cannot pass
adapter removing process
              SeqFile_too_short_reads.fa ---seqs that are lower than
required minimal length threshold
              SeqFile_too_long_reads.fa ---seqs that are higher than
required maximal length threshold
              -SeqFile_match_genome.fa
                                               ---segs that can match to
reference genome
             -SeqFile_unmatch_genome.fa
                                             ---seqs that cannot match to
reference genome
              SeqFile_match_<X>_match_genome.fa ---seqs that can match to
both <X> database and reference genome
              SeqFile_match_<X>_unmatch_genome.fa ---seqs that can match to
<X> database but not reference genome
              -SeqFile unmatch <X> match genome.fa
                                                  ---segs that cannot match
to <X> database but can match to reference genome
              LSeqFile_unmatch_<X>_unmatch_genome.fa ---seqs that match to
<X> rfam database nor reference genome
```

```
SeqFile_processed (if -k applied)
             ---seqs that match to
reference genome in BOWTIE format
             ---seqs that match to both
miRNA database and reference genome in BOWTIE format
             LSeqFile_output_<X>_unmatch_genome ---seqs that match to
miRNA database but not reference genome in BOWTIE format
         LSeqFile_result
             SeqFile_output.txt ---6 column table file including
annotation information for every unique sequence
             ---3 column table file including
reads number of each major- (e.g. rRNA) and sub- (e.g. 5S rRNA) classes
             ---3 column table file
including reads number of each length distribution of each major class
             SeqFile_mismatch_summary.txt ---11 column table file
including mismatch statistics information for each mismatch loci (if -M >0)
             ---figure of length
distribution of miRNA, rsRNA, tsRNA, piRNA and other RNAs, if sequence matches
existed
             -SeqFile_rRNA_distribution.pdf
                                               ---figure of length
distribution of different types of rRNAs, if sequence matches existed
             -SeqFile_rRNA_mapping.pdf
                                            ---figure of rsRNAs mapping
against different types of rRNAs, if sequence matches existed
             LSeqFile_tRNA_mapping.pdf
                                           ---figure of length
distribution of different types of tRNAs, if sequence matches existed
      ├processing_report (if -k applied)
         L<sub>1</sub> SeqFile.txt
                                   ---processing log file
      └sh file (if -k applied)
         L<sub>1</sub> SeqFile.sh
                                   ---processing script file
```

```
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
```

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 all the sequences. In this case, the abundance of this sequence is the highest.

'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: <SPORTS_output_fold_address>

- 3. Output:
 - 1. <seq_fold>_output.txt: A 6 column table file including annotation information for every unique sequence.

```
Sequence
                                              Length Reads
Match_Genome Annotation
   t00000001 TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGC 32 1234567
            tRNA-Glu-CTC_5_end
   ______
   -ID: t00000001
                                             --Represents the unique
ID of the sequence, represents the abundance ranking among all the
sequences. In this case, the abundance of this sequence is the highest.
   -Sequence: TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGC --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 reference genome.
   -Annotation: tRNA-Glu-CTC_5_end
                                            --The annotation of the
sequence. This sequence mapped against the 5' end of tRNA-Glu-CTC sequence.
```

2. <seq_fold>_summary.txt: A 3 column table file including reads number of each major- and sub-classes.

```
-Sub_Class: tRNA-Glu-CTC_5_end --The sub class name.
-Reads: 123456 --The reads number of the class.
```

3. <seq_fold>_length_distribution.txt: A 3 column table file including reads number of each length distribution of each major class.

```
Class
                                     Length
                                               Reads
   tRNAdb-tRNA_5_end_Match_Genome
                                     30
                                               1234
                                               23456
   tRNAdb-tRNA_5_end_Match_Genome
                                     31
   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 of the sequence.
   -Length: 30
                                             -- The reads number of the
   -Reads: 1234
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_length>
```

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_name

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/EvZP50IfGFRJINJwZRujI00BM-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/guestaccess.aspx? docid=0773ed3d5f6b74f35bbd643e1af221c31&authkey=AcRxf8walnGUIEhgl--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.ncrna
-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/guestaccess.aspx? docid=03a9a8d26cca14b458007e9c6ee4541f7&authkey=Aag33OX-ljvagRWePhYNF3k)
 - 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Gorilla_gorilla/UCSC/gorGor5/Sequence/BowtieInd
ex/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/guestaccess.aspx? docid=03a74e9f6c2594f1e86a31acd8e554621&authkey=AYrgOm8rrAY7hrFYQ03gmTA)
- 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/guestaccess.aspx? docid=09c13507ee7414365843de3450aa9ad3e&authkey=AdsLOHA5q2--SbiP2C6Qjpc)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Pan_troglodytes/UCSC/panTro4/Sequence/BowtieInd
ex/genome
-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.ncrna
-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/guestaccess.aspx? docid=037168296877942ecb9735f26afcb450a&authkey=AZkU5ib3A0KOiT4KxrhgTGQ)
- 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/guestaccess.aspx? docid=068ef6629d31b4fb28100f667050be1d1&authkey=AWEPi3HUmOVD_PPPnkMhdvs)
- 6.2 SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Nomascus_leucogenys/UCSC/nomLeu3/Sequence/BowtieInde
x/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/guestaccess.aspx? docid=07fededf7468444ba9b863b74316b8504&authkey=Aa6X06J4ExLvKtH8mJ-CESs)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Macaca_mulatta/UCSC/rheMac8/Sequence/BowtieInde
x/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/guestaccess.aspx? docid=03a22a2092c7b46fb93a8fc49cf234720&authkey=Ad_hzH3MUIMh0-9BLoH_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/guestaccess.aspx? docid=077698888fa8d40408df8c979e91146e4&authkey=AeoDYI5a3lKyF-CWgupu6IA)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Callithrix_jacchus/UCSC/calJac3/Sequence/Bowtie
Index/genome
-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/guestaccess.aspx? docid=0c761313792b64396b87a66a6e04101be&authkey=AZBsjTA5-hXdASOlimxNL4I)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Carlito_syrichta/UCSC/tarSyr2/Sequence/BowtieIn
dex/genome
-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/guestaccess.aspx? docid=0b2cacd8453104b2abb60298863fc4c16&authkey=AZAYeCOsLKuc_ml-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.Rno
r_6.0.ncrna
-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/guestaccess.aspx? docid=0833653a140eb47f098267d7a23d3b63c&authkey=Ab8aoYC8paqFl2yRablo7Ok)
- 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.ncrna
-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/guestaccess.aspx? docid=0b0ac3830026f4007958774cbdb421632&authkey=AZnURBxU1PYzUO1yyrcoZ_M)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Cricetulus_griseus/UCSC/criGri1/Sequence/Bowtie
Index/genome
-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/guestaccess.aspx? docid=0d27261e6ae9c4402bbc9465addb544de&authkey=Ad1xlk56DNm0StozUWDqCYw)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Cavia_porcellus/UCSC/cavPor3/Sequence/BowtieInd
ex/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/guestaccess.aspx? docid=0ceff76c620884395899bcd385bfaa098&authkey=ATiBTnSjWKqY0zPKQOmM6kU)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Heterocephalus_glaber/UCSC/hetGla2/Sequence/Bow
tieIndex/genome
-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/guestaccess.aspx? docid=0419ea57bd00548cc94574d8ce9717fcd&authkey=Aa1pkb9R7850Ss3GFmB6GzM)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ictidomys_tridecemlineatus/UCSC/speTri2/Sequenc
e/BowtieIndex/genome
-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/guestaccess.aspx? docid=068d8d8388b74c38943e50237a259a88&authkey=AemP73yDCDiObexlk-DcRVQ)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ochotona_princeps/UCSC/ochPri3/Sequence/BowtieI
ndex/genome
-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/guestaccess.aspx? docid=01b2d69333c97448196cc69b212e92fc9&authkey=ARbwyo0-WJX10nDdXwvNygc)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Oryctolagus_cuniculus/UCSC/oryCun2/Sequence/Bow
tieIndex/genome
-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/guestaccess.aspx? docid=0e880ae29a16747bebf5b9afdb5956921&authkey=AYIP0UkTwtVuiul45XA7mYE)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ovis_aries/UCSC/oviAri3/Sequence/BowtieIndex/ge
nome
-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/guestaccess.aspx? docid=0a58e56dc7cb743299631fec15b72e69d&authkey=AeosGslpVMdvtZa6qtKJBCE)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Bos_taurus/UCSC/bosTau8/Sequence/BowtieIndex/ge
nome
-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)

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=0e15d39ac05b24a9b899e7a9dfcf96773&authkey=AYaNWQ9KLlkqq7f2qbWzchc)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Sus_scrofa/UCSC/susScr3/Sequence/BowtieIndex/ge
nome
-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/guestaccess.aspx? docid=08396ad3619a247d695508aee771e3991&authkey=AVKuiimDuoOGVKfGvZcK_ik)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Tursiops_truncatus/UCSC/turTru2/Sequence/Bowtie
Index/genome
-t /<your_defined_address>/Tursiops_truncatus/GtRNAdb/turTru2-tRNAs
-f /<your_defined_address>/Tursiops_truncatus/Rfam_12.3/Rfam-12.3-
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/guestaccess.aspx? docid=0d27ad173ffdb4dcc8a4954f9ba5426eb&authkey=AUdYvbA-_g0lzVEEBlth8V8)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Balaenoptera_acutorostrata/UCSC/balAcu1/Sequenc
e/BowtieIndex/genome
-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/guestaccess.aspx? docid=0633e2d7781ab4aefb59fc03f1347657b&authkey=AXrP4XnyiHQqkC5WOUhhS5w)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Erinaceus_europaeus/UCSC/eriEur2/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=0399046c56fb246c39630c84db61a23ad&authkey=AWE7pAlGt1TAAUylD4qoE9A)
- 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/guestaccess.aspx?

docid=06a45d60105a04796b5e96a9417d86f4c&authkey=AY37yxKX-C5u9DG71tzrFEI)

2. SPORTS1.1 related parameters if you download recommended reference database (both UCSC and Ensembl genome sequences are provided):

```
-g
/<your_defined_address>/Canis_familiaris/UCSC/canFam3/Sequence/BowtieIn
dex/genome

or
-g /<your_defined_address>/Canis_familiaris/Ensembl/Cfam_1.0/dna/genome

-m /<your_defined_address>/Canis_familiaris/miRBase/22.1/miRBase-cfa
-r /<your_defined_address>/Canis_familiaris/rRNAdb/dog_rRNA
-t /<your_defined_address>/Canis_familiaris/GtRNAdb/canFam3-tRNAs
-e
/<your_defined_address>/Canis_familiaris/Ensembl/Cfam_1.0/rna/Canis_lup
us_familiaris.ROS_Cfam_1.0.rna
-f /<your_defined_address>/Canis_familiaris/Rfam/14.7/Rfam
```

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/guestaccess.aspx? docid=0ef4e067d42f241359e2e606b14e0d8f9&authkey=AUW2yr7SM356KpD5uhBNOeM)
- 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/guestaccess.aspx? docid=0215799b172f94c909c5251061e317540&authkey=AdE82hkEi1MHI3OL1vY92b8)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ailuropoda_melanoleuca/UCSC/ailMel1/Sequence/Bo
wtieIndex/genome
-t /<your_defined_address>/Ailuropoda_melanoleuca/GtRNAdb/ailMel1-tRNAs
-f /<your_defined_address>/Ailuropoda_melanoleuca/Rfam-12.3-panda
```

29. Felis catus (Cat)

 annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=0203fe1a0c8954879878ef8cabcfe37cf&authkey=AZ32jdZI7FoFU_t8_NFb9o0)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Felis_catus/UCSC/felCat8/Sequence/BowtieIndex/g
enome
-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/guestaccess.aspx? docid=0e7011a73d57545ea8be3c71335b3cf4c&authkey=AWHkBfbzVmwz_HjmQXo8IJU)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Equus_caballus/UCSC/equCab2/Sequence/BowtieInde
x/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/guestaccess.aspx? docid=0f836e7106664459bacda6f94dc15e22d&authkey=AVSh1b1dr84q53sLTqD9sRA)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ceratotherium_simum/UCSC/cerSim1/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=03dcc351bbc274cfc8692e5f2b1f5d0f3&authkey=AbjlxSozqu1c-2sRUrTGn7k)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Myotis_lucifugus/UCSC/myoLuc2/Sequence/BowtieIn
dex/genome
-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/guestaccess.aspx? docid=06448172c574b4b9cbe0d906cf75bc68b&authkey=AX_fEuPmr18NZPEFbcg9nEQ)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Trichechus_manatus/UCSC/triMan1/Sequence/Bowtie
Index/genome
-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/guestaccess.aspx? docid=0e0146e32fe4745bba50bbede409efddc&authkey=AT8bNP2DhjKysDaGD4Qy-7s)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Loxodonta_africana/UCSC/loxAfr3/Sequence/Bowtie
Index/genome
-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/guestaccess.aspx? docid=0c68adc6b2afc447a9dfe45a1a5eedd49&authkey=AUPTS51dr88E3AMvcZtljrk)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Dasypus_novemcinctus/UCSC/dasNov3/Sequence/Bowt
ieIndex/genome
-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)

- 1. annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=076db8740d2e34caabb25669fd6297e36&authkey=AWv-dFi1Mm7C0QH9K00WHH4)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Notamacropus_eugenii/UCSC/macEug2/Sequence/Bowt
ieIndex/genome
-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/guestaccess.aspx? docid=0469dc3788cbb40bab7472ee70a230dc0&authkey=AXA1nDb4QpYeuIJFC-D6mL0)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Sarcophilus_harrisii/UCSC/sarHar1/Sequence/Bowt
ieIndex/genome
-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/guestaccess.aspx? docid=05e9cd2f3891a4761ade95d98aa3ebc78&authkey=Ae_jBKldBC7HypVTN7S98Rw)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Monodelphis_domestica/UCSC/monDom5/Sequence/Bow
tieIndex/genome
-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_domes
tica.BROADO5.ncrna
-f /<your_defined_address>/Monodelphis_domestica/Rfam_12.3/Rfam-12.3-
opossum
```

39. Ornithorhynchus anatinus (Platypus)

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=07f0f4ed088844a86afa5db763407699c&authkey=AUFx9yWXHtg1CQc-wfpJ81M)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Ornithorhynchus_anatinus/UCSC/ornAna2/Sequence/
BowtieIndex/genome
-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/Ornithorhynchu
s_anatinus.OANA5.ncrna
-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/guestaccess.aspx? docid=0defbf712045f4e7f85b373b0eba4cd1b&authkey=AbieXkq6akbsD8tY1oGctDI)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Taeniopygia_guttata/UCSC/taeGut2/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=0e0046ab3b1a9458a9a183b45507ea0c3&authkey=AWiCroVnVDQcPENZDX_76VM)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Melopsittacus_undulatus/UCSC/melUnd1/Sequence/B
owtieIndex/genome
-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/guestaccess.aspx? docid=0abafb326c4074fe9971d60a26497126c&authkey=AcRw_9ltjRXbdNAfmLRl_gg)
- 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_gall
us-5.0.ncrna
-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/guestaccess.aspx? docid=0c88e1661f08e4b6d945d87e8120bdf07&authkey=AWICjj414nXNuNbrNM7mbE4)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Meleagris_gallopavo/UCSC/melGal1/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=0fbd62d91eb4442a88525f89684a74242&authkey=AY3fgBdvPRzWpqqD4yd_Sqw)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Chrysemys_picta/UCSC/chrPic1/Sequence/BowtieInd
ex/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/guestaccess.aspx? docid=06986df31c45c4a00a6a2b47ce4ee7af2&authkey=AaE-i58-1fjJeqcDHZUpLLo)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Anolis_carolinensis/UCSC/anoCar2/Sequence/Bowti
eIndex/genome
-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
.AnoCar2.0.ncrna
-f /<your_defined_address>/Anolis_carolinensis/Rfam_12.3/Rfam-12.3-
lizard
```

46. Xenopus laevis (Frog)

 annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=0d9b3d45b3bf3483c826c36d2b340f0fd&authkey=AdGZ969RHd1lpwBpm7lsGEQ)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Xenopus_laevis/UCSC/xenTro7/Sequence/BowtieInde
x/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/guestaccess.aspx? docid=0be8104aeb59d4ff89909d0c62d0a2f4e&authkey=AasK4DZcbB12a8wB8CNz6Ak)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Latimeria_chalumnae/UCSC/latCha1/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=06252eaadd5894a3795afce052716fd17&authkey=AWZ9FVt-iphiQFRDzuKoJtA)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Tetraodon_nigroviridis/UCSC/tetNig2/Sequence/Bo
```

```
wtieIndex/genome
-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/guestaccess.aspx? docid=026501a5c4aa54930a00289acf9691f5f&authkey=AWUaDYW0_VZx-1dy5wnCfdQ)
- 2. 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/guestaccess.aspx? docid=0268db779d3654712b39d5450bac55894&authkey=AQwh0wJeAcvF78JRu4RHddM)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Gasterosteus_aculeatus/UCSC/gasAcu1/Sequence/Bo
wtieIndex/genome
-t /<your_defined_address>/Gasterosteus_aculeatus/GtRNAdb/gasAcu1-tRNAs
-e
/<your_defined_address>/Gasterosteus_aculeatus/Ensembl/Gasterosteus_acu
leatus.BROADS1.ncrna
-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/guestaccess.aspx? docid=0f5089e2cc47245f28dca1a3f8f75343c&authkey=AV8N2wRviWth_LjhJqpB0zk)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Oryzias_latipes/UCSC/oryLat2/Sequence/BowtieInd
ex/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/guestaccess.aspx? docid=0cf791d1d99de4f4c8ed7f8de6d5694f8&authkey=AZZgbe3d1aKb7GDuCDlv81w)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Oreochromis_niloticus/UCSC/oreNil2/Sequence/Bow
tieIndex/genome
-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)

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=08f5a65a279f34d518cc4017cb04fe469&authkey=ASMQLlwmWReD_bVW922N0lo)
- 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/guestaccess.aspx? docid=07d3448d6cd29485498e70f8f067a619d&authkey=Aa1-VjshX-GKLZI7limHFlo)
- 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)

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=053976e5c17b9435982d22223b9389eba&authkey=ASL1VUPU4Ol2PskRXpj94t4)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Callorhinchus_milii/UCSC/calMil1/Sequence/Bowti
eIndex/genome
-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/guestaccess.aspx? docid=013a1f7a5585b462b801f1cfe3faf2cdd&authkey=AVN94xcHd1_Aa2ofYovsw8Q)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Petromyzon_marinus/UCSC/petMar2/Sequence/Bowtie
Index/genome
-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/guestaccess.aspx? docid=0840567689dbe4ff49852c8744056f172&authkey=AWhmaQmPgN5fuB1RXIKc69U)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Strongylocentrotus_purpuratus/UCSC/strPur2/Sequ
ence/BowtieIndex/genome
-m
/<your_defined_address>/Strongylocentrotus_purpuratus/miRBase_21/miRBas
e_21-spu
-r
/<your_defined_address>/Strongylocentrotus_purpuratus/rRNA_db/urchin_rR
NA
-t /<your_defined_address>/Strongylocentrotus_purpuratus/GtRNAdb/Spurp-
tRNAs
-e
/<your_defined_address>/Strongylocentrotus_purpuratus/Ensembl/Strongylo
centrotus_purpuratus.GCA_000002235.2.ncrna
-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/guestaccess.aspx? docid=06d15ec2032e141648ce4eedd413b0e0c&authkey=ARejQLC8ofAhQq9lwwoB0Pw)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Drosophila_melanogaster/UCSC/dm6/Sequence/Bowti
eIndex/genome
-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_mela
nogaster.BDGP6.ncrna
-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/guestaccess.aspx? docid=003bfc542d06b42edb24a94969bb12b36&authkey=AdgdWQ5emWRWTYvXcgPT0Fo)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Anopheles_gambiae/UCSC/anoGam1/Sequence/BowtieI
ndex/genome
-m /<your_defined_address>/Anopheles_gambiae/miRBase_21/miRBase_21-aga
-t /<your_defined_address>/Anopheles_gambiae/GtRNAdb/Agamb-tRNAs
-e
/<your_defined_address>/Anopheles_gambiae/Ensembl/Anopheles_gambiae.Aga
mP4.ncrna
-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/guestaccess.aspx? docid=0877c09c8493b47e9b9fb6c2c0bdc6015&authkey=AQJrbZlyZz9MDnPB9uYxMYE)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Pristionchus_pacificus/UCSC/priPac1/Sequence/Bo
wtieIndex/genome
-m
/<your_defined_address>/Pristionchus_pacificus/miRBase_21/miRBase_21-
ppc
-r
/<your_defined_address>/Pristionchus_pacificus/rRNA_db/roundworm_rRNA
-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):

- annotation database: (We provide a download link for all databases listed below: https://ncrnainfo-my.sharepoint.com/personal/sports_ncrna_info/_layouts/15/guestaccess.aspx? docid=0cffdc7d5a3844567bc5303adca47fb81&authkey=AdA1jCVLRJkz2k8oSVU-pCI)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Caenorhabditis_elegans/UCSC/ce10/Sequence/Bowti
eIndex/genome
-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_e
legans.WBcel235.ncrna
-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/guestaccess.aspx? docid=0a974d08ffcb842dea8d18462dcfaf2f4&authkey=AQ6wsr9hNwVfCfqvuScKStE)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Saccharomyces_cerevisiae/UCSC/sacCer3/Sequence/
BowtieIndex/genome
-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_
cerevisiae.R64-1-1.ncrna
-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/guestaccess.aspx? docid=0abdce07adf9449e89bdcb89d4d4609a4&authkey=AViNEp3y6Y4hNBYSEuW_sYg)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Zea_mays/Ensembl/AGPv4/Sequence/BowtieIndex/gen
ome
-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/guestaccess.aspx? docid=0335fb9b05a024e61819c04db7ffd2a51&authkey=AdMT31NWtny7F1NzJNn3zZI)

2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Sorghum_bicolor/Ensembl/Sbi1/Sequence/BowtieInd
ex/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_v2.ncrna
-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/guestaccess.aspx? docid=0510818bde4a342868928f982420dd07c&authkey=ASfsIUL0BX2HYew7xrw2x1A)
- 2. SPORTS1.1 related parameters if you download recommend reference database:

```
-g /<your_defined_address>/Oryza_sativa/Ensembl/IRGSP-
1.0/Sequence/BowtieIndex/genome
-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/guestaccess.aspx? docid=08ec312d8fc7d4211952170468dfbb202&authkey=AeVag9p4ifZJG58C5UbXstE)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Arabidopsis_thaliana/Ensembl/TAIR10/Sequence/Bo
wtieIndex/genome
-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_thalia
na.TAIR10.ncrna
-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/guestaccess.aspx? docid=079974655bb2e4f62b669c3d1701fffeb&authkey=AWVriz_LNaTMDsBjbMXDS60)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Glycine_max/Ensembl/Gm01/Sequence/BowtieIndex/g
enome
-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/guestaccess.aspx? docid=0645cc2a0024d41fdba5be31a17bd5374&authkey=AaR4ui2QEXqh2-SpOlxaUik)
- 2. SPORTS1.1 related parameters if you download recommended reference database:

```
-g
/<your_defined_address>/Escherichia_coli/Ensembl/EB1/Sequence/BowtieInd
ex/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.HUSEC
2011CHR1.ncrna
-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
GTCTACGGCCATACCACCCTGAACGCGCCCGATCTCGTCTGATCTCGGAAGCTAAGCAGGGTCGGGCCTG
GTTAGTACTTGGATGGAGACCGCCTGGGAATACCGGGTGCTGTAGGCTTT

human rRNA 5.8S.fa

>Human 5.8S ribosomal RNA
CGACTCTTAGCGGTTGGATCACTCGGCTCGTGCGTCGATGAAGAACGCAGCGCTAGCTGCGAGAATTAATG
TGAATTGCAGGACACATTGATCATCGACACTTCGAACGCACTTGCGGCCCCGGGTTCCTCCCGGGGCTAC
GCCTGTCTGAGCGTCGCTT

- 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 mitocondrial tRNA sequences from mitotRNAdb (http://mttrna.bioinf.uni-leipzig.de/mtDataOutput/) in .fa format and reformat the file X_mito-tRNA.fa by using the scripts provided below and put the generated files X-mt_tRNA.fa and X-mt_tRNA_CCA.fa into the defined folder address: <your_defined_address>; (optional)

mt tRNA transfer.pl X mito-tRNA.fa X-mt tRNA

- 6. 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)
- 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

1.1.2 - 12/25/2024

- 1. Added comprehensive instructions for running the demo small RNA-seq data analysis.
- 2. Included the demo output results generated by SPORTS1.1.

1.1.1 - 03/10/2021

- 1. Now the parameters performed in the pipeline will be dislayed in the header of the output log;
- 2. Fixed some bugs in the previous version.

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 (the bowtie index will build when the user using SPORTS for the first time);
- 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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