sRNAtoolbox Manual

sRNAtoolbox aims to provide small RNA researchers with several useful tools including sRNA expression profiling from deep sequencing experiments and several downstream analysis tools. The centrepiece of sRNAtoolbox is sRNAbench (the successor program of miRanalyzer which was originally published in 2009), which allows for expression profiling from deep sequencing experiments. Either the other tools can be launched on sRNAbench results, or independently using the appropriate file formats.

Currently the toolbox comprises the following methods:

- sRNAbench
- sRNAde
- sRNAblast
- sRNAcons
- miRNAconsTarget: animalconstarget and plantconstarget

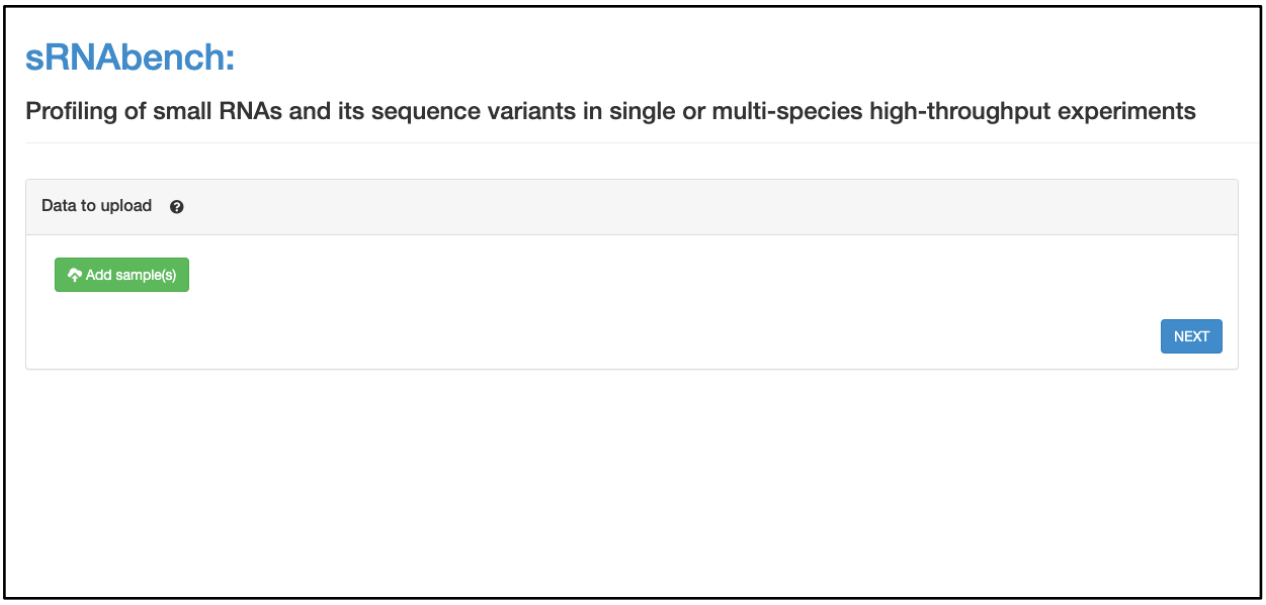
sRNAbench

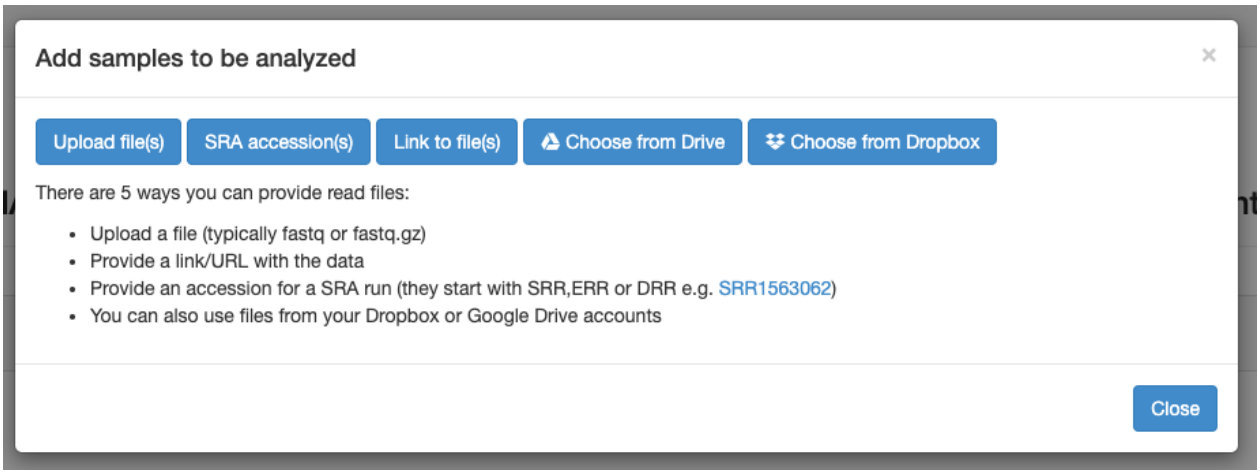
Profiling of small RNAs and their sequence variants in single or multi-species high-throughput experiments

Description

Several commonly used input formats like fastq, sra, fasta or read/count are supported. Input files can be provided via upload, URL, SRA accession, Google Drive or Dropbox. sRNAbench performs expression profiling of known microRNAs and other types of small RNAs, detection and analysis of isomiRs, outputs several graphical summaries and predicts novel microRNAs in both plants and animals. Result example

Sequencing data input page





The sequencing data can be provided in 5 different ways:

- Upload one or several files (typically fastq or fastq.gz)
- Provide one or several link/URL(s) with the data (it will be downloaded and then profiled)
- Provide one/several accession(s) for SRA runs (they start with SRR, ERR or DRR e.g. SRR1563062)
- Choose files from your Google Drive account
- Choose files from your Dropbox account

To add samples, simply click on the desired button and then add the files using the prompt or add the SRA accessions/URLs (one per line) and click on the “ADD” button.
Once all your read files have been provided, you can click “Next” to move on to the parameter specification.

Traditionally, we have supported an unlimited number of samples to be uploaded but we may revisit this policy if some users start engaging in abuse of these conditions.

Example on how to use SRA data

The following link will take us to the SRA project page which contains all information provided by the authors (see below)

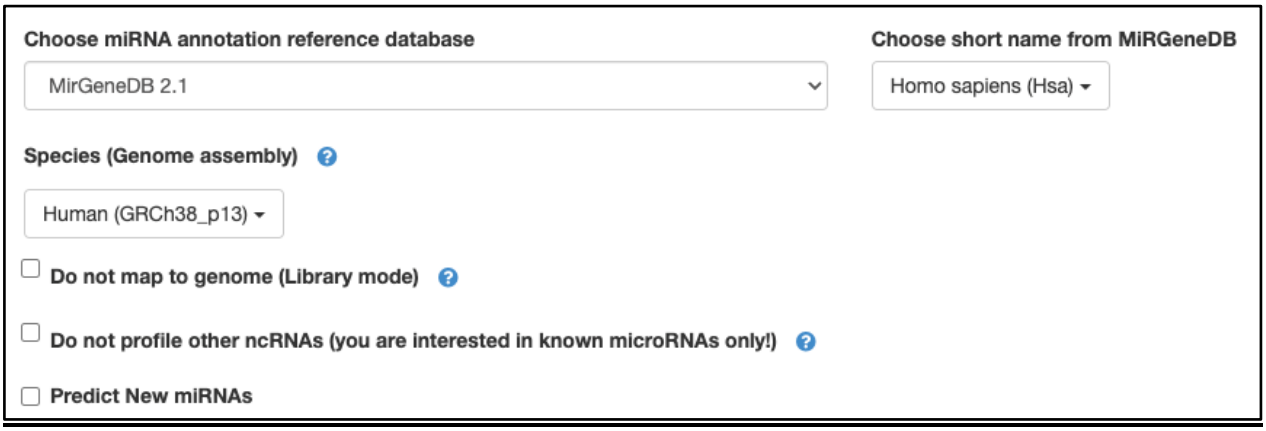
12 Runs found

Run	BioSample	Sample name	DATASTORE provider	DATASTORE location	AvgSpotLen	Experiment	LoadDate	MBases	MBytes	disease
SRR1563064	SAMN03015564	RN sRNA exosome fraction	ncbi		101	SRX691181	2014-10-03	765	534	
SRR1563063	SAMN03015563	RN sRNA cell fraction	ncbi		101	SRX691180	2014-10-03	951	666	
SRR1563062	SAMN03015562	IM-1 sRNA exosome fraction	sra-sos	sra-sos.public	100	SRX691179	2014-09-05	184	109	
SRR1563061	SAMN03015561	IM-1 sRNA cell fraction	sra-sos	sra-sos.public	100	SRX691178	2014-09-05	773	455	
SRR1563060	SAMN03015320	IK140508 sRNA exosome fraction	ncbi		101	SRX691177	2014-10-03	597	417	
SRR1563059	SAMN03015319	IK140508 sRNA cell fraction	ncbi		101	SRX691176	2014-10-03	572	397	
SRR1563058	SAMN03015312	MUTU I clone 9 sRNA exosome fraction	sra-sos	sra-sos.public	100	SRX691175	2014-09-05	799	464	Burkitt Lymphoma 9
SRR1563057	SAMN03015311	MUTU I clone 9 sRNA cell fraction	sra-sos	sra-sos.public	100	SRX691173	2014-09-05	472	261	Burkitt Lymphoma 9
SRR1563056	SAMN03015312	MUTU I clone 9 sRNA exosome fraction	ncbi		100	SRX691171	2014-10-03	1,139	697	Burkitt Lymphoma 9
SRR1563018	SAMN03015308	MUTU I clone 3 sRNA cell fraction	sra-sos	sra-sos.public	100	SRX691160	2014-09-05	714	399	Burkitt Lymphoma 3
SRR1563017	SAMN03015315	BJAB sRNA exosome fraction	ncbi		100	SRX691158	2014-10-03	584	301	Diffuse Large B-cell Lymphoma
SRR1563015	SAMN03015313	BJAB sRNA cell fraction	ncbi		101	SRX691157	2014-10-03	1,284	873	Diffuse Large B-cell Lymphoma

This table can be downloaded and the SRR identifiers can be extracted and pasted into the corresponding textbox (see below).

Input parameters page

Select species annotation:



Alignment of small RNA reads **requires** at least a miRNA reference annotation or genomic annotations. The left side dropdown selector allows you to choose the miRNA reference database (MirGeneDB or PmiREN are recommended if available), the right side selector allows you to choose one or several species of interest. Then the remaining selector will let you choose genome assembly and annotations from Ensembl for one or several species of interest.
You can decide not to map to the genome (slightly faster but miRNA/sRNA loci cannot always be distinguished), not to profile to other ncRNAs or if you want to predict novel miRNAs from your sequencing data (which requires a genome assembly). This prediction will be performed after all miRNA and other ncRNA reads have been assigned.

Reads preprocessing:

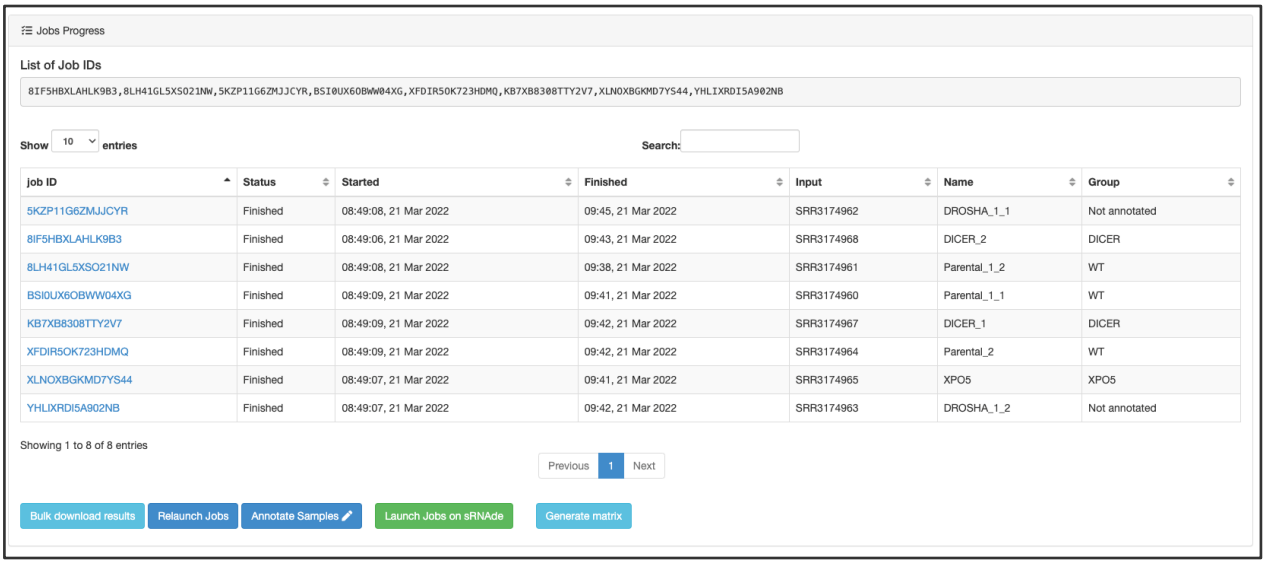
Reads preprocessing: we have implemented five different library preparation protocols for straightforward adapter removal. If your experiment was not designed using these five protocols, custom settings can still be selected. If reads are already trimmed, it can also be specified here.

Optional Parameters

- Quality Control: There are two methods to filter out reads of low quality: ***mean-based***, a read is accepted if the mean Phred score is above a given threshold; and ***minimum per nucleotide***, a read is eliminated if a single position of the read has a Phred score below the given threshold. The Phred threshold can be defined by the user.

- Parameters: other parameters that can be adjusted like bowtie mapping parameters and minimum read counts or maximum and minimum read lengths.

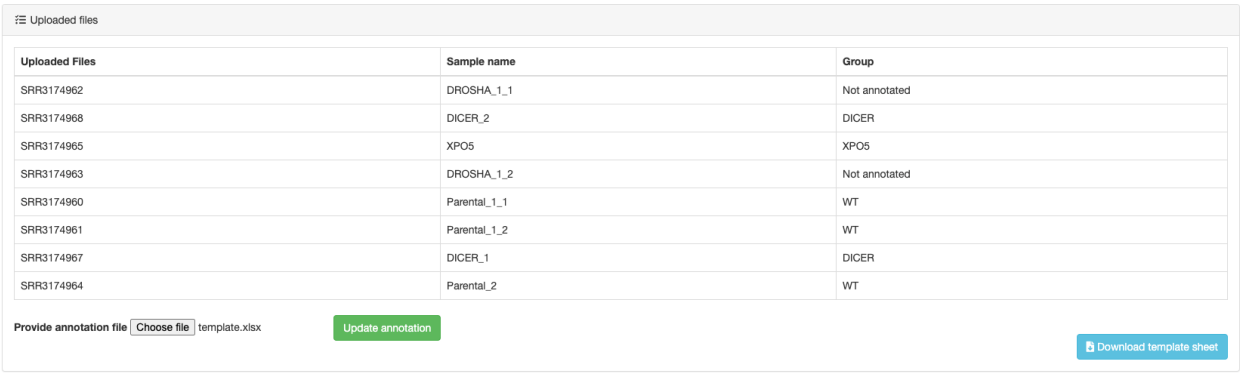
Status page



Samples launched will be listed in the status page. In this table users can check information about the status of their job, start and finish time, what input was used and name or group information (if provided) as well as a link to navigate to the individual result of each sample. To provide sample names and groups for differential expression (once all jobs are done running) you can click on the button “Annotate Samples”. This will take you to a different page where samples can be annotated using an excel file. Other functions include bulk zipped download of all results, job relaunching (sample samples with different parameters), Differential Expression (Launch sRNAde) and Matrix Generation.

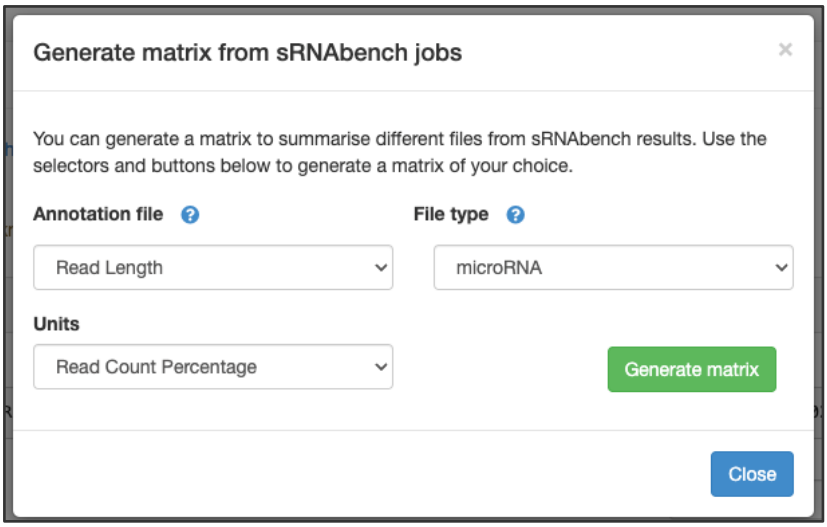
- Launch jobs on sRNAde: Once all sRNAbench jobs are finished aligning, the user can launch an sRNAde job to perform differential expression. Users can navigate to a new page where they can manually provide groups/conditions for their samples or they can use annotations previously provided using the “Annotate Samples” button.

- Annotate samples:



Users can easily annotate their samples using this feature which provides a pregenerated template (Download template file) with their samples and sample names or sample groups if they had previously been annotated. If a sample is not assigned to a group (i.e. left blank) it will not be taken into consideration for the differential expression analysis.

- Generate matrix:



By clicking on the “Generate matrix” button users can access this menu where different files from sRNAbench can be summarized into a matrix. For some matrices, a visualization is also available.

Individual job output page

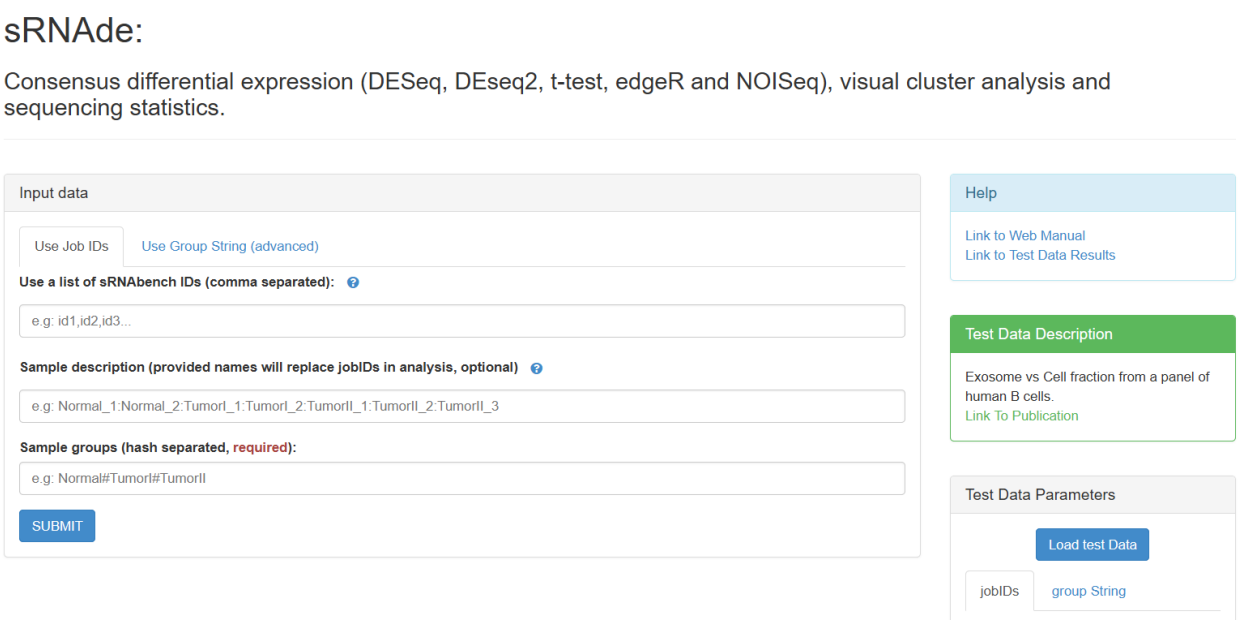
By clicking on the link of each sRNAbench job, users can access a report for each individual sample. There are five sections in the sRNAbench report:

- Parameters: a display of all the parameters used to run the job including sequencing protocol, genome/species and input file.
- Summary: a series of preprocessing and mapping statistics/plots that can provide an overview of the quality of the sequencing library and of the mapping.
- Genome mapping: a summary of the reads mapping to genome and unique reads.
- microRNA summary: a summary of reads mapping to miRNAs (selected reference and genomes) including plots and links to tables to individually explore each mature miRNA sequence, hairpin align files and isomiR distribution.
- sRNA summary: similarly to the previous section, abundance tables are provided for each sRNA category both using single and multiple assignment.
- Other features: this tab is designed to contain current and future functionalities that do not fall within any previous section. Right now, it contains anticodon statistics of tRNAs present in the profile.

Besides these sections, users can download a zip file containing all the result files of the sRNAbench command line tool (check the tool manual for more details). Feel free to contact us if you cannot find a file you were expecting in there or if there is some information you would like to see included.

sRNAde

Consensus differential expression (DESeq, edgeR and NOISeq), visual cluster analysis and sequencing statistics.



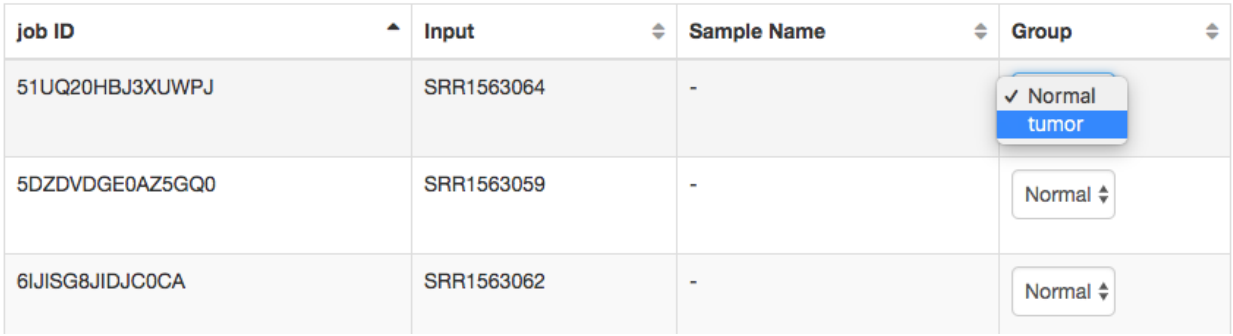
Description

sRNAde is a module for the detection of differentially expressed sRNA. The input is either a number of sRNAbench output folders or a user-given expression matrix. For the detection of differentially expressed RNAs it applies three widely used methods: edgeR, DESeq and NOISeq. Apart of the output from the individual methods, it provides a consensus differential expression file. Additionally, this tool can perform cluster analysis (heat maps), isomiR analysis and it gives a summary on the sequencing statistic of all used samples (if the input has been from sRNAbench). Result example

Input

Users should provide a list of sRNAbench jobIDs on which differential expression analysis of miRNAs will be performed. There are two ways to do this:

- A list of jobIDS and group names: On the "Use Job IDs" tab the user can provide a list of sRNAbench job IDs (comma separated) and group names (comma separated, as many as the user wants to include in the analysis). In a second step, each sample can be assigned to the group it belongs using a dropdown selector (see image below)



a. A list of colon and hash jobIDs using # to separate groups (GroupString): job IDs should be provided in the following way:

f1_1:f2_1#f1_2:f2_2 being f1_1 the ID of the first sample of the first group (controls in a case/control study), f2_1 the second sample of the first group, f1_2 the first sample of the second group, etc. That means that groups are separated by hashes (#), while samples are separated by colon (*:*).

Note, the ID is assigned by sRNAbench and can be found either in the URL or in the output page

For example, the test data run can also be launched using this grpString:
D42PPR3ZVTVQBK8:EYHC83BJX80ATIL:UWCUSRT3IZO3ME2:XXEYZ4EZ55BHKUG:YH5LQJZVNP9W3NX:5DZDVDGE0AZ5GQ0#51UQ20HBJ3XUWPJ:6IJISG8JIDJC0CA:FKZ2D88FZNWZCS5:OE1ZNRX2RSJ0OV1:OUSQYGT9V0V6ZA5:ZBM4WYEO1C80DTU

and the following group description string:
exosome#cell
The test data comprises therefore 2 experimental groups (exosome and cell). sRNAde calculates the differential expression between all possible combinations (only exosome vs cell in this case).

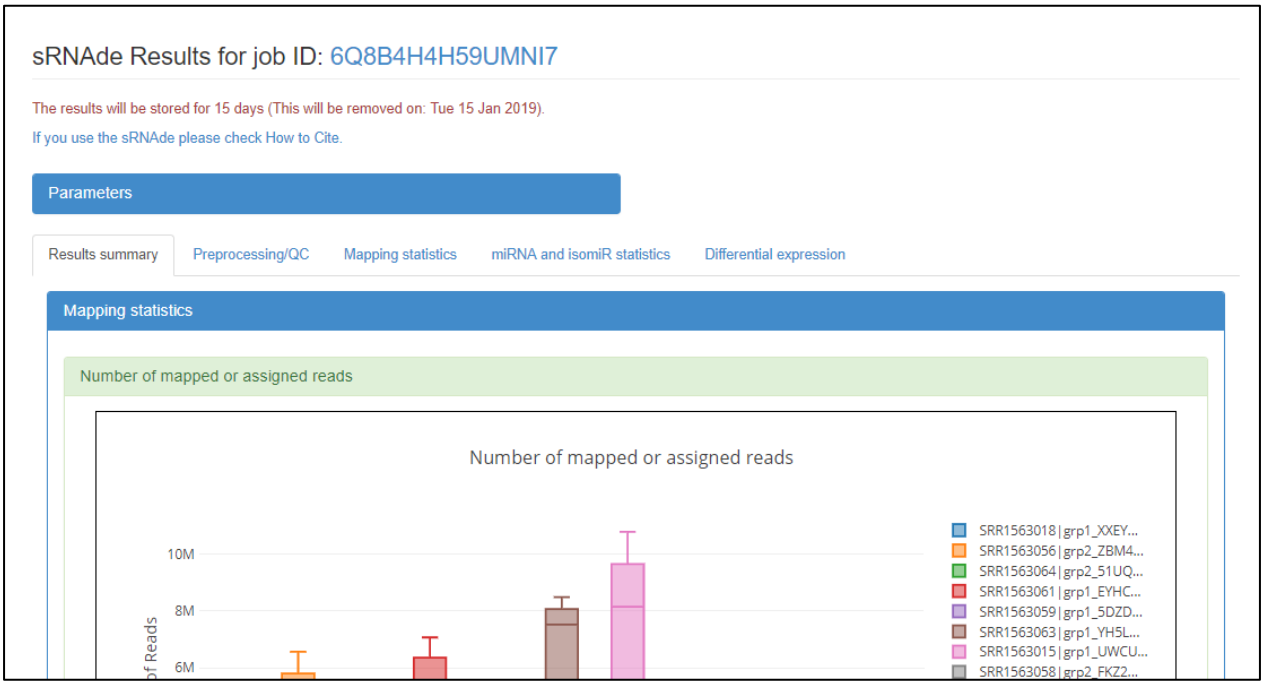
The user can also provide a description of the samples separated by colons. If no description is given, the sRNAbench job name is used by default.

Finally, the user can provide differential expression thresholds for NOISeq, DESeq, DEseq2, t-test and edgeR.

Please, note they are all adjusted p-value.

Additionally the user can enable isomiR analysis, checking the box “isomiR Analysis”.

Results



b. **Results summary**: Mapping statistics of reads.
    a. Number of mapped or assigned reads
    b. Percentage of mapped or assigned reads
    c. Percentage per detected RNA category
    d. Detected number of miRNAs and precursor sequences

c. **Preprocessing/QC**: Statistics of reads preprocessing and quality control.
    a. Preprocessing statistics
    b. Read length distribution (full, analysis and genome mapped)

d. **Mapping statistics**: The user can visualize the genome mapping distribution.

e. **miRNA and isomiR statistics**: The user can visualize the fraction of different isomiR classes

per sample.

f. **Differential expression**: We provide the result of differential expression of the 5 methods for each comparison between groups defined by user. In this section, only significantly differentially expressed miRNAs are shown (the cut-off value can be provided by the user). The result description of each method is widely explained in their manuals:
    a. NOISeq
    b. DESeq
    c. DESeq2
    d. EdgeR

sRNAblast

Profiling of small RNAs of unknown origin

Description

This tool is intended for the analysis of reads that could not be mapped using sRNAbench or other profiling tools. The results could point towards either contamination sources or biological meaningful information like the presence of unexpected viral or bacterial RNA molecules. Result example

Input

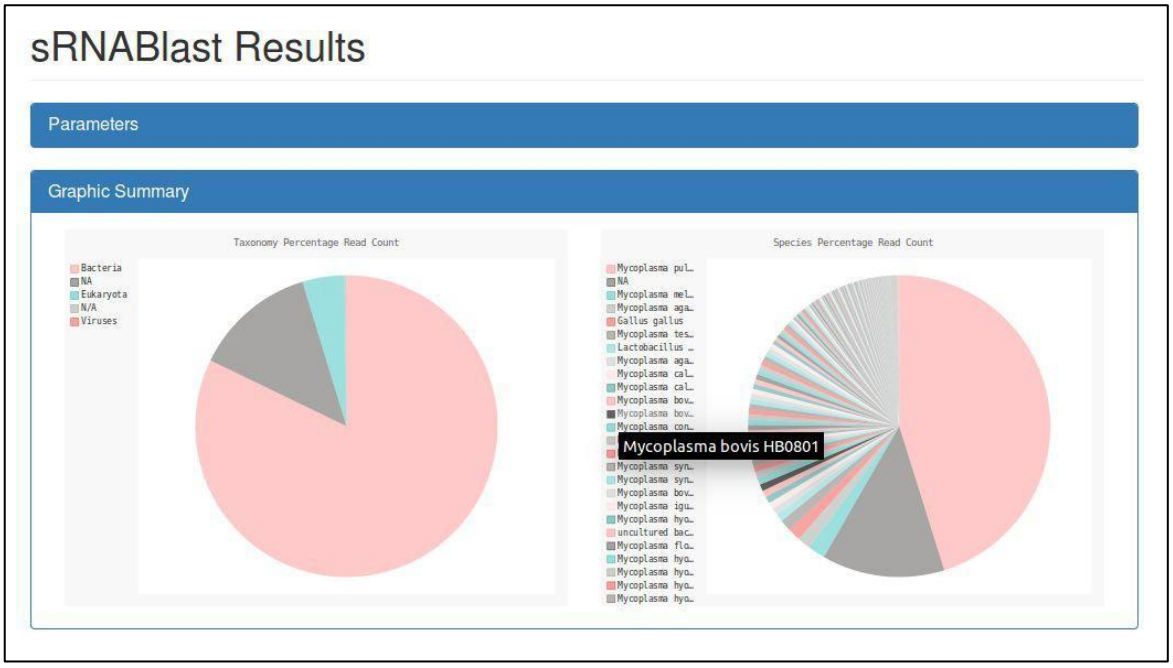
The datasets must be provided uploading a file from a local computer or by means of an *URL*. It accepts the same input formats as sRNAbench. In general, all formats can be compressed with gzip. Additionally a sRNAbench ID can be used as input - in this case, only the unmapped reads

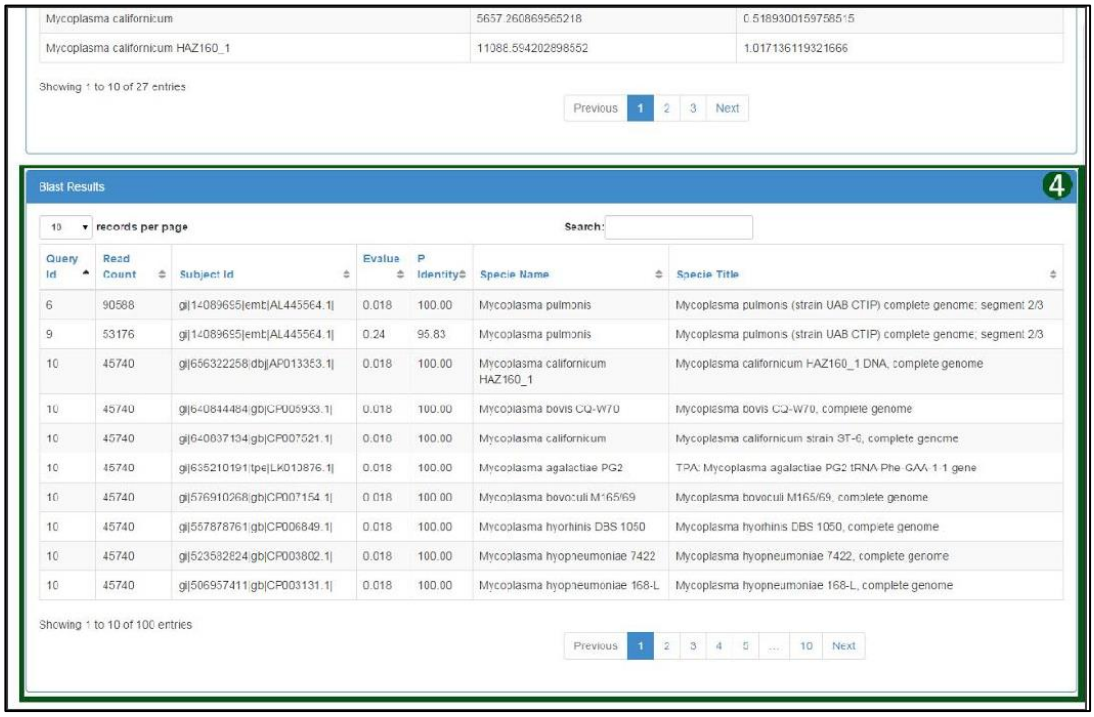
are used, i.e. to determine the origin of those unmapped reads.

Adapter Removal

sRNAblast can perform the adapter trimming. The web-server version will by default search for the first 10 bases of the adapter allowing a maximum of one mismatches. It is recommended to provide the adapter sequence or select one of the options given by the application, which are the most common adapters used on microRNA analysis: Illumina RA3, Illumina (alternative) or SOLiD (SREK). If the adapter is not known, although it is not recommended, guess the adapter sequence option should be activated. Then, sRNAblast will align the first 250,000 reads to the genome using the bowtie seed functionality (the adapters will not count for the mismatches).

Out of all aligned reads, the adapter sequence is defined as the most frequent 10-mer starting at the first mismatch. Lastly, when the adapter is sequenced at the very end of the read, sometimes its length is shorter than the length threshold, so it must be search in a recursively way without taking into account the minimum length.





Results

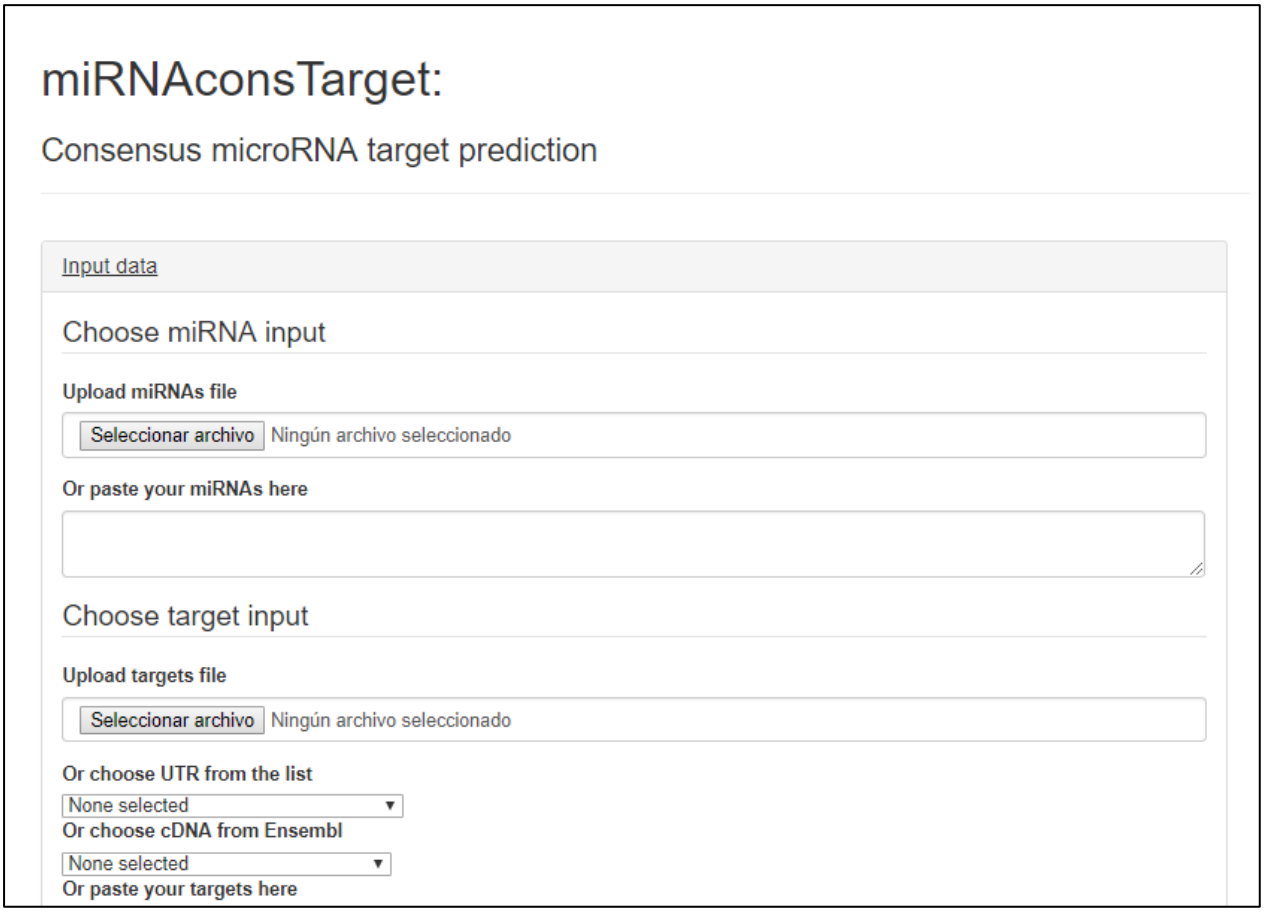
1. This table provide the number of reads detected in each taxonomic group:
    a. Taxonomy: Taxonomy group Name
    b. Read Count: Number of reads detected in this taxonomic group
    c. Percentage Read Count: Percentage of reads detected in this taxonomic group
2. This table provide the number of reads detected in each specie.
    a. Species: Species Name
    b. Read Count: Number of reads detected for this species
    c. Percentage Read Count: Percentage of reads detected for this species
3. In this section, the full result can be downloaded as zip file.
4. This table contain the number of reads for each blast result:
    a. Query id: Query id
    b. Read count: Number of reads in this subject sequence
    c. Subject id: Subject id
    d. Evalue: Blast e-value
    e. Percentage of Identity: Blast percentage of identity value
    f. Specie Name: Subject specie
    g. Specie Title: NCBI Subject Title

miRNAconsTarget: animal- and plantconstarget

Predict microRNA targets on user defined sets of microRNAs and 3'UTRs

Description

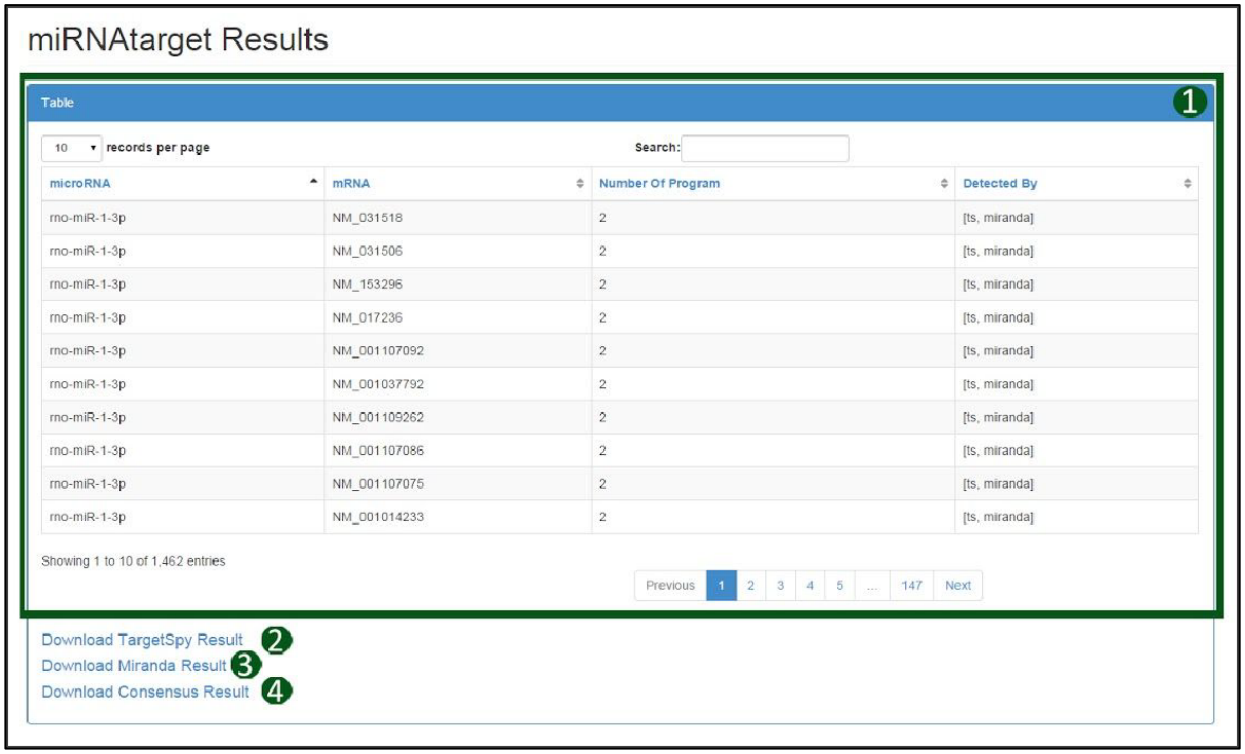
Most microRNA target prediction tools are implemented as webservers on a limited number of species, or do allow only the prediction of a limited number of microRNAs and/or mRNAs. miRNAconsTargets uses three microRNA target prediction programs for animals (PITA, miRanda and TargetSpy) and 2 for plants (psRobots and TAPIR) and applies them to the user supplied sets of microRNAs and 3'UTRs. It reports both, the individual predictions and a consensus prediction. Result example



Input

The input files (miRNAs file and utr files) must be provided uploading a file in fasta format. If the user wants to detect plant microRNA target genes, the 'Is Plant Analysis' checkbox must be activated. Three options can be chosen for animals and three for plants. Note that TAPIR can be

used in RNAhybrid mode and fasta (alignment) mode.



Results

1. In this table consensus result are shown:
    a. miRNA: miRNA name
    b. mRNA: transcript Target name
    c. Number of Programs: Number of programs that predicted this mRNA as target
    d. Detect by: Names of programs that predicted this mRNA as target

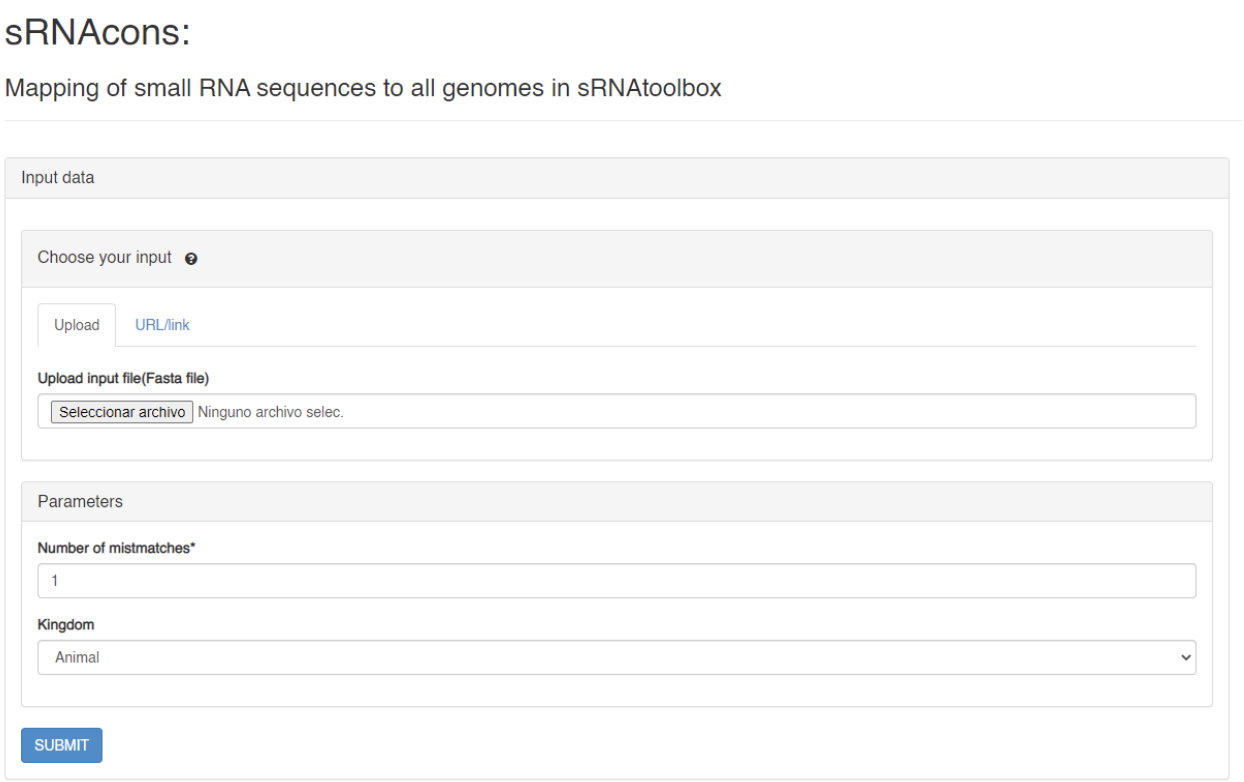
2. Link to download TargetSpy results
3. Link to download miRanda results
4. Link to download Consensus results

sRNAcons

Estimates the conservation depth of a set of small RNAs

Description

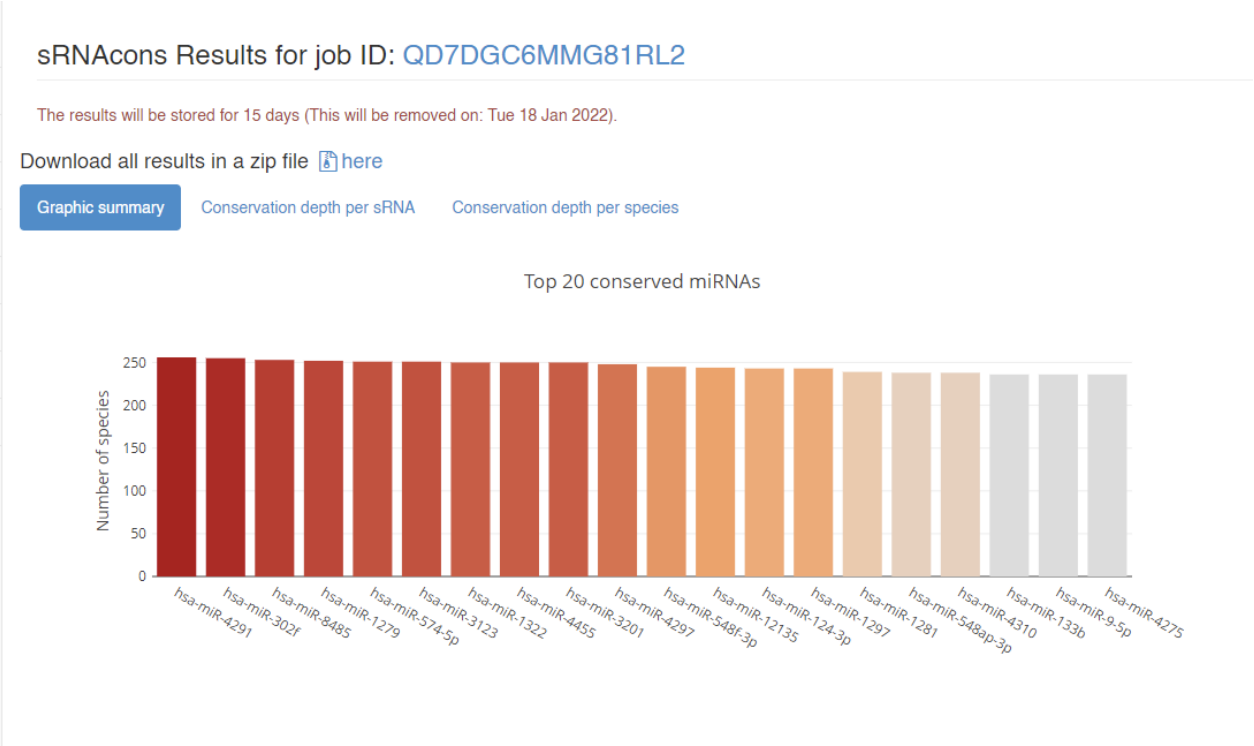
User provided small RNA sequences (fasta format) are mapped against all assemblies available in sRNAtoolbox. The tool has two different outputs, i) The conservation depth for all small RNA input sequences, i.e the percentage of genomes in which the sequence was found, and ii)the percentage of mapped input sequences per genome.



Input

The input file should be a fasta file (provided updating the file of by means of an *URL*) with the small RNA sequences of which you want to estimate conservation depth (for example a set of mature miRNAs). You can also choose the number of mismatches you want to allow in the sequences alignment, and the kingdom (animal or plants) against which you want to align your

sequences.



Results

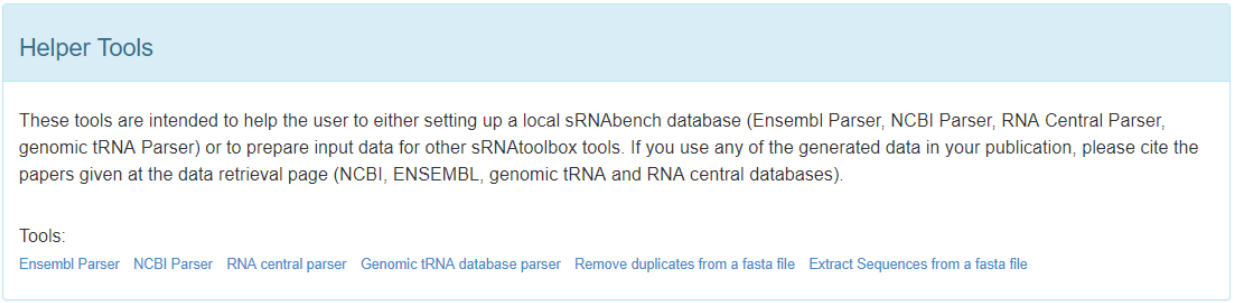
1. **Graphic summary**:
    a. Top 20 conserved miRNAs
    b. Top 20 species with the highest number of conserved sRNAs
2. **Conservation depth per sRNA :** The user can visualize/download a table with the conservation depth per sRNA sequence, including the frequency and a list of species in which the sequence is conserved per sRNA.
3. **Conservation depth per species:** The user can visualize/download a table with the frequency of the input sRNAs per species.

Helper Tools

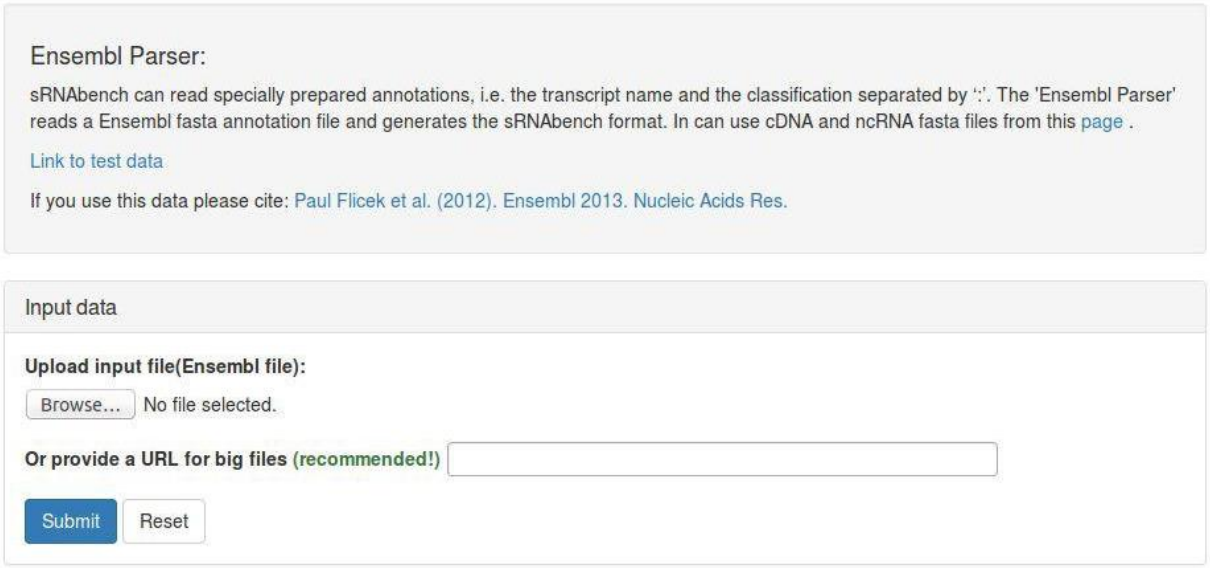
sRNAtoolbox implements several ‘helper tools’ which are intended to aid the generation of a local sRNAbench database or/and which might be useful for the preparation of input data of some of

the tools. Below, the six different helper tools are described in more detail.

- Ensembl Parser
- NCBI Parser
- RNA central parser
- Genomic tRNA database parser
- Remove duplicates from a fasta file
- Extract Sequences from a fasta file



Ensembl Parser



NCBI Parser

NCBI Parser:

sRNAbench can read specially prepared annotations, i.e. the transcript name and the classification separated by ':'. The 'NCBI Parser' reads a NCBI fasta annotation file and generates the sRNAbench format. In can use *.rna.fna.gz files from the following page .

Link to test data

If you use this data please cite: Kim D. Pruitt et al. (2012). NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. Nucleic Acids Res. PMCID: PMC3245008.

Input data

Upload input file(NCBI file):

Browse... No file selected.

Or provide a URL for big files (recommended!)

Submit Reset

RNA central parser

RNA central parser:

This parser extracts the non-coding sequences of a given species or taxonomy level from the RNA central database version 2 (February 2015) (http://rnacentral.org/) and prepares the sRNAbench format libraries.

Test with: Hordeum vulgare

If you use this data please cite: The RNAcentral Consortium (2014) RNAcentral: an international database of ncRNA sequences. doi: 10.1093/nar/gku991

Input data

Provide a Species Name (Must be Scientific Name):

Or provide a Taxonomy Name:

Submit Reset

Genomic tRNA database parser

RNA central parser:

tRNA annotations can be obtained parsing a species out of the genomic tRNA database (http://gtrnadb.ucsc.edu/).

Test with: Homo sapiens

If you use this data please cite: Chan, P.P., Lowe, T.M. (2009) GtRNAdb: A database of transfer RNA genes detected in genomic sequence Nucl. Acids Res. 37(Database issue):D93-D97.

Input data

Provide a Species Name (Must be Scientific Name):

Submit Reset

Remove duplicates from a fasta file

Remove Duplicates from a Fasta File :

The UCSC table browser allows to obtain 3' UTR sequences which are needed when searching for microRNA target genes. However the output files have the following format:

```
>hg19_refGene_NM_001184906 range=chr17:37408897-37417712 5'pad=0 3'pad=0 strand=- repeatMasking=none
CAATGGAGGTGGTCAACCTTGGCGAACTGAGTATTTAATGACACTTCTAG
AGCTACCGTGGAGTCTCTCCAGTGGAAGCAACCCCAGTGTTCTGAGCAAG
```

The name of the sequence ' hg19_refGene_NM_001184906' would not be recognized by downstream analysis programs (functional enrichment analysis). This parser allows to substitute certain parts of the sequence name in order to recover the name of the transcript (NM_001184906 in this case).

Specifying 'hg19_refGene_' in the textbox would make the parser to remove this string from the sequence name leaving only the name of the transcript. This parser removes also duplicated IDs and gives the additional possibility to remove duplicated sequence.

Input data

Upload input file(Fasta file):

Browse... No file selected.

Or provide a URL for big files (recommended!)

Provide a string of characters to be drop out from the sequence names:

Remove also duplicate SEQUENCES

Submit Reset

Extract Sequences from a fasta file