miRge3

Release 0.0.1

Arun H. Patil and Marc K. Halushka

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An update to Python package to perform comprehensive analysis of small RNA sequencing data, including miRNA annotation, A-to-I editing, novel miRNA detection, isomiR analysis, visualization through IGV, processing Unique Molecular Identifieres (UMI), tRF detection and producing interactive graphical output.

miRge3.0 is developed in python v3.8 and is a recent update of our previous version miRge2.0. This build includes command line interface (CLI) and cross-platform Graphical User Interface (GUI). For more details refer to documentation link below.

CHAPTER

ONE

LINKS

- Documentation
- Source code
- Report an issue
- Project page on PyPI

CHAPTER

TWO

TABLE OF CONTENTS

2.1 Installation

2.1.1 Linux OS

Welcome to installation protocol for Linux OS

Install python3.8 and R

This installation protocol is based on Ubuntu, please use the commands that suit your Linux distribution. For example, `apt` should be replaced with `yum` in Fedora/CentOS.

- Search and start the terminal
- Follow the commands to update Ubuntu and install python 3.8 A password will be prompted when you type `sudo`, use the one you have set during Ubuntu (or your distro) installation.

```
sudo apt update
sudo apt install software-properties-common
sudo add-apt-repository ppa:deadsnakes/ppa
sudo apt install python3.8
sudo apt install python3-setuptools
sudo apt install python3-pip
sudo apt install r-base
```

Linux (Ubuntu 18.04) comes with python2.7 installed by default. To use python3.8, creating an alias in .bashrc would do the trick.

Use vim editor if you are familiar using this editor `vi .bashrc` or open the .bashrc using text editor by `gedit .bashrc` and add the following line at the bottom of the text. `alias python=python3.8`

Save and exit. After that type `bash` on the command line -Or- simply, close the terminal.

Installing miRge3.0

First install miRge dependenceis

- Search and start the terminal, execute the command below:

```
python3.8 -m pip install --user cutadapt==2.7 reportlab==3.5.42 biopython==1.77
↪scikit-learn==0.23.1  hypothesis==5.15.1 pytest==5.4.2  scipy==1.4.1  matplotlib==3.
↪2.1  joblib==0.15.1  pandas==1.0.3 future==0.18.2
```

If you encounter a `WARNING`, like below:

```
WARNING: The script cutadapt is installed in '/home/arun/.local/bin' which is not on
↪PATH.
  Consider adding this directory to PATH or, if you prefer to suppress this warning,
↪use --no-warn-script-location.
```

Then, open a new terminal window or type `cd` to get to home directory. Add `bin` folder PATH to the .bashrc, as shown below: Example: `export PATH=$PATH:"/home/arun/.local/bin"` Remeber to add your path `/PATH_TO_USERS/bin`.

Install miRge3.0 by this simple command

```
python3.8 -m pip install --user mirge3
```

To upgrade miRge3.0

```
python3.8 -m pip install --user --upgrade  mirge3
```

Install additional C-libraries based tools

Install Bowtie

- Search and start the terminal
- Download bowtie

```
wget -O bowtie-1.2.3-macos-x86_64.zip  https://sourceforge.net/projects/bowtie-bio/
↪files/bowtie/1.2.3/bowtie-1.2.3-macos-x86_64.zip/download
```

- `unzip bowtie-1.2.3-macos-x86_64.zip`
- `cd bowtie-1.2.3-macos-x86_64`
- `pwd`
  - `/home/arun/software/bowtie-1.2.3-linux-x86_64`
- Add these bowtie binaries to .bashrc as shown below:

```
export PATH=$PATH:"/home/arun/software/bowtie-1.2.3-linux-x86_64"
```

- After that type `bash` on the command line -Or- simply, close the terminal.

Install Samtools

- Search and start the terminal, execute the below command: `sudo apt install samtools`

Install RNA Fold

- Search and start the terminal, execute the following commands:
- wget "https://www.tbi.univie.ac.at/RNA/download/sourcecode/2_4_x/ViennaRNA-2.4.16.tar.gz"
- cd ViennaRNA-2.4.16

```
sudo ./configure
sudo make
sudo make install
```

GUI requirements

Providing system wide access to miRge3.0, cutadapt, bowtie and bowtie-build, please type or (copy and paste) and submit each of the following commands on the terminal: **NOTE:** Make sure to change your path to python bin folder; Replace `/home/arun/.local/` with `/Path on your computer/`.

- Search and start the terminal, execute the following commands:

```
sudo ln -s /home/arun/.local/bin/miRge3.0 /usr/local/bin/miRge3.0
sudo ln -s /home/arun/.local/bin/cutadapt /usr/local/bin/cutadapt
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie /usr/local/bin/bowtie
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie-build /usr/local/bin/
↪bowtie-build
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie-inspect /usr/local/
↪bin/bowtie-inspect
```

Downloading FASTQ files from NCBI:

- Search and start the terminal, follow the commands below:
- wget -c https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/2.10.8/sratoolkit.2.10.8-mac64.tar.gz
- tar -xvzf sratoolkit.2.10.8-mac64.tar.gz
- cd sratoolkit.2.10.8-mac64/bin
- `pwd`
  - `/home/arun/software/sratoolkit.2.10.8-ubuntu64/bin`
- Add to .bashrc
  - `cd`
  - `vi .bashrc` or `gedit .bashrc` and add the following line at the bottom of the page
  - `export PATH=$PATH:"/home/arun/software/sratoolkit.2.10.8-ubuntu64/bin"`

Save and exit. After that type `bash` on the command line -Or- simply, close the terminal.

Obtaining and installing GUI application

- Download GUI for Linux

Uninstalling miRge3.0

To uninstall open the terminal and type:

```
python3.8 -m uninstall mirge3
```

2.1.2 macOS

Welcome to installation protocol for Mac OS

System prerequisites

- Search and start the terminal, execute the following commands
- `ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/master/install)"`
- `brew update`
- `brew install wget`

Install python3.7

Please note, any version other than py3.7 causes error in Mac with multiprocessing, issues-1, issues-2. Download python 3.7.5 from python.org

- Search and start the terminal, execute the following commands

```
wget https://www.python.org/ftp/python/3.7.5/python-3.7.5-macosx10.9.pkg
sudo installer -pkg python-3.7.5-macosx10.9.pkg -target /
```

Mac comes with python2.7 installed by default. To use python3.7, creating an alias in .bash_profile would do the trick Open a new terminal window. Use vim editor if you are familiar using this editor `vi .bash_profile` or open the .bash_profile using text editor by `open -e .bash_profile` and add the following line at the bottom of the text.

```
alias python=python3.7
```

Save and exit. After that type `source ~/.bash_profile` on the command line -Or- simply, close the terminal.

Install R

- Search and start the terminal, execute the following command

```
brew install r
```

Installing miRge3.0

First install miRge dependenceis

- Search and start the terminal, execute the following command

```
python3.7 -m pip install --user cutadapt==2.7 reportlab==3.5.42 biopython==1.77
↪scikit-learn==0.23.1  hypothesis==5.15.1 pytest==5.4.2  scipy==1.4.1  matplotlib==3.
↪2.1  joblib==0.15.1  pandas==1.0.3 future==0.18.2
```

If you encounter a `WARNING`, like below:

```
WARNING: The script cutadapt is installed in '/Users/loaneruser/Library/Python/3.7/bin
↪' which is not on PATH.
  Consider adding this directory to PATH or, if you prefer to suppress this warning,
↪use --no-warn-script-location.
```

Then, open a new terminal window or type `cd` to get to home directory. Add `bin` folder PATH to the .bash_profile, as shown below: Example: `export PATH=$PATH:"/Users/loaneruser/Library/Python/3.7/bin/"` Remeber to add your path `/PATH_TO_USERS/Python/3.7/bin`.

Install miRge3.0 by this simple command

```
python3.7 -m pip install --user  mirge3
```

To upgrade miRge3.0

```
python3.7 -m pip install --user --upgrade  mirge3
```

Install additional C-libraries based tools

Install Bowtie

- Search and start the terminal, execute the following command
- Download bowtie

```
wget -O bowtie-1.2.3-macos-x86_64.zip  https://sourceforge.net/projects/bowtie-bio/
↪files/bowtie/1.2.3/bowtie-1.2.3-macos-x86_64.zip/download
```

- `unzip bowtie-1.2.3-macos-x86_64.zip`
- `cd bowtie-1.2.3-macos-x86_64`
- `pwd`
  - `/Users/loaneruser/Software/bowtie-1.2.3-macos-x86_64`
- Add these bowtie binaries to .bash_profile as shown below:

```
export PATH=$PATH:"/Users/loaneruser/Software/bowtie-1.2.3-macos-x86_64/"
```

- After that type `source ~/.bash_profile` on the command line -Or- simply, close the terminal.

Install Samtools

- Search and start the terminal, execute the following command `brew install samtools`

Install RNA Fold

- wget "https://www.tbi.univie.ac.at/RNA/download/sourcecode/2_4_x/ViennaRNA-2.4.16.tar.gz"
- cd ViennaRNA-2.4.16

```
sudo ./configure
sudo make
sudo make install
```

Downloading FASTQ files from NCBI:

- Search and start the terminal, execute the following command
- wget -c https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/2.10.8/sratoolkit.2.10.8-mac64.tar.gz
- tar -xvzf sratoolkit.2.10.8-mac64.tar.gz
- cd sratoolkit.2.10.8-mac64/bin
- `pwd`
  - `/Users/loaneruser/Software/sratoolkit.2.10.8-mac64/bin`
- Add to .bash_profile
  - `cd`
  - `vi .bash_profile` or `open -e .bash_profile` and add the following line at the bottom of the page
  - `export PATH=$PATH:"/Users/loaneruser/Software/sratoolkit.2.10.8-mac64/bin"`

Save and exit. After that type `source ~/.bash_profile` on the command line -Or- simply, close the terminal.

GUI requirements

Providing system wide access to miRge3.0, cutadapt, bowtie and bowtie-build, please type or (copy and paste) and submit each of the following commands on the terminal: **NOTE:** Make sure to change your path to python bin folder; Replace `/Users/loaneruser/Library/` with `/Path on your computer/`.

- Search and start the terminal, execute the following command

```
sudo ln -s /Users/loaneruser/Library/Python/3.7/bin/miRge3.0 /usr/local/bin/miRge3.0
sudo ln -s /Users/loaneruser/Library/Python/3.7/bin/cutadapt /usr/local/bin/cutadapt
sudo ln -s /Users/loaneruser/Software/bowtie-1.2.3-macos-x86_64/bowtie /usr/local/bin/
↪bowtie
sudo ln -s /Users/loaneruser/Software/bowtie-1.2.3-macos-x86_64/bowtie-build /usr/
↪local/bin/bowtie-build
sudo ln -s /Users/loaneruser/Software/bowtie-1.2.3-macos-x86_64/bowtie-inspect /usr/
↪local/bin/bowtie-inspect
```

Obtaining and installing GUI application

- Download GUI for OSX

Uninstalling miRge3.0

To uninstall open the terminal and type:

```
python3.8 -m uninstall mirge3
```

2.1.3 Windows OS

Welcome to installation protocol for Windows OS

System prerequisites

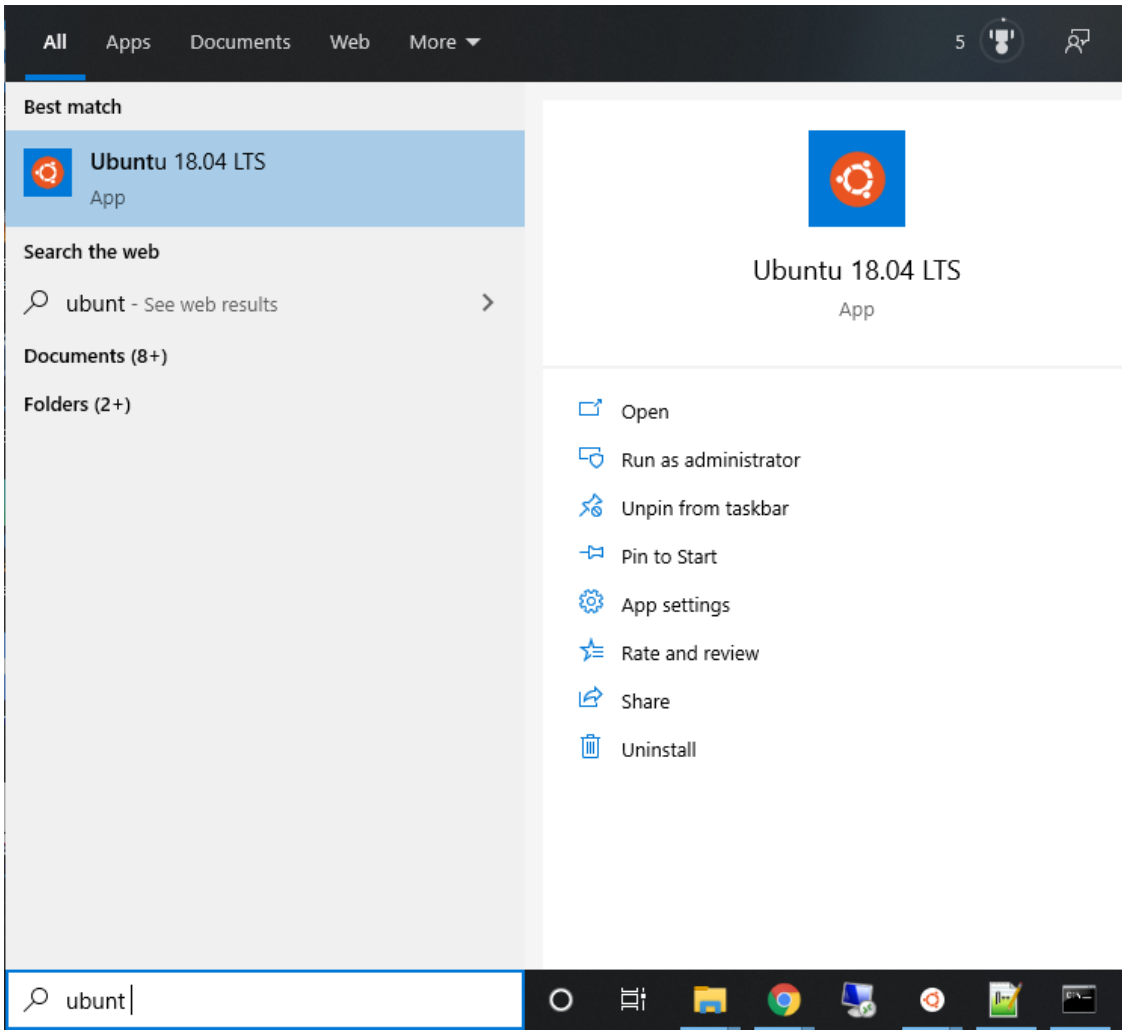
- `Require Windows 10`
- `Require WSL and Ubuntu 18`

Install WSL

Please follow one of the following guidlines for installing WSL and Ubuntu 18.04 (recommended Ubuntu distribution)

- Quick and easy way
  - TopTechSkills: Watch the first 1:30 seconds, more info.
  - Patreon: Watch the first 4:04 seconds.
- Official windows page.
- `Please remember the password prompted during ubuntu installation` and use when prompted.

Install python3.8 and R



- Search and start Ubuntu
- Follow the commands to update ubuntu and install python 3.8 A password will be prompted when you type `sudo`, use the one you have set during Ubuntu installation.

```
sudo apt update
sudo apt install software-properties-common
sudo add-apt-repository ppa:deadsnakes/ppa
sudo apt install python3.8
sudo apt install python3-setuptools
sudo apt install python3-pip
sudo apt install r-base
```

Linux (Ubuntu 18.04) comes with python2.7 installed by default. To use python3.8, creating an alias in .bashrc would do the trick

Use vim editor if you are familiar using this editor `vi .bashrc` or open the .bashrc using text editor by `gedit .bashrc` and add the following line at the bottom of the text. `alias python=python3.8`

Save and exit. After that type `bash` on the command line -Or- simply, close the terminal.

Installing miRge3.0

First install miRge dependenceis

- Search and start Ubuntu, execute the following command

```
python3.8 -m pip install --user cutadapt==2.7 reportlab==3.5.42 biopython==1.77
↪scikit-learn==0.23.1  hypothesis==5.15.1 pytest==5.4.2  scipy==1.4.1  matplotlib==3.
↪2.1  joblib==0.15.1  pandas==1.0.3 future==0.18.2
```

If you encounter a `WARNING`, like below:

```
WARNING: The script cutadapt is installed in '/home/arun/.local/bin' which is not on
↪PATH.
  Consider adding this directory to PATH or, if you prefer to suppress this warning,
↪use --no-warn-script-location.
```

Then, open a new terminal window or type `cd` to get to home directory. Add `bin` folder PATH to the .bashrc, as shown below: Example: `export PATH=$PATH:"/home/arun/.local/bin"` Remeber to add your path `/PATH_TO_USERS/bin`.

Install miRge3.0 by this simple command

```
python3.8 -m pip install --user mirge3
```

To upgrade miRge3.0

```
python3.8 -m pip install --user --upgrade  mirge3
```

Install additional C-libraries based tools

Install Bowtie

- Search and start Ubuntu, execute the following command
- Download bowtie

```
wget -O bowtie-1.2.3-macos-x86_64.zip  https://sourceforge.net/projects/bowtie-bio/
↪files/bowtie/1.2.3/bowtie-1.2.3-macos-x86_64.zip/download
```

- `unzip bowtie-1.2.3-macos-x86_64.zip`
- `cd bowtie-1.2.3-macos-x86_64`
- `pwd`
  - `/home/arun/software/bowtie-1.2.3-linux-x86_64`
- Add these bowtie binaries to .bashrc as shown below:

```
export PATH=$PATH:"/home/arun/software/bowtie-1.2.3-linux-x86_64"
```

- After that type `bash` on the command line -Or- simply, close the terminal.

Install Samtools

- Search and start Ubuntu, execute the following command `sudo apt install samtools`

Install RNA Fold

- wget "https://www.tbi.univie.ac.at/RNA/download/sourcecode/2_4_x/ViennaRNA-2.4.16.tar.gz"
- cd ViennaRNA-2.4.16

```
sudo ./configure
sudo make
sudo make install
```

GUI requirements

Providing system wide access to miRge3.0, cutadapt, bowtie and bowtie-build, please type or (copy and paste) and submit each of the following commands on the terminal: **NOTE:** Make sure to change your path to python bin folder; Replace `/home/arun/.local/` with `/Path on your computer/`.

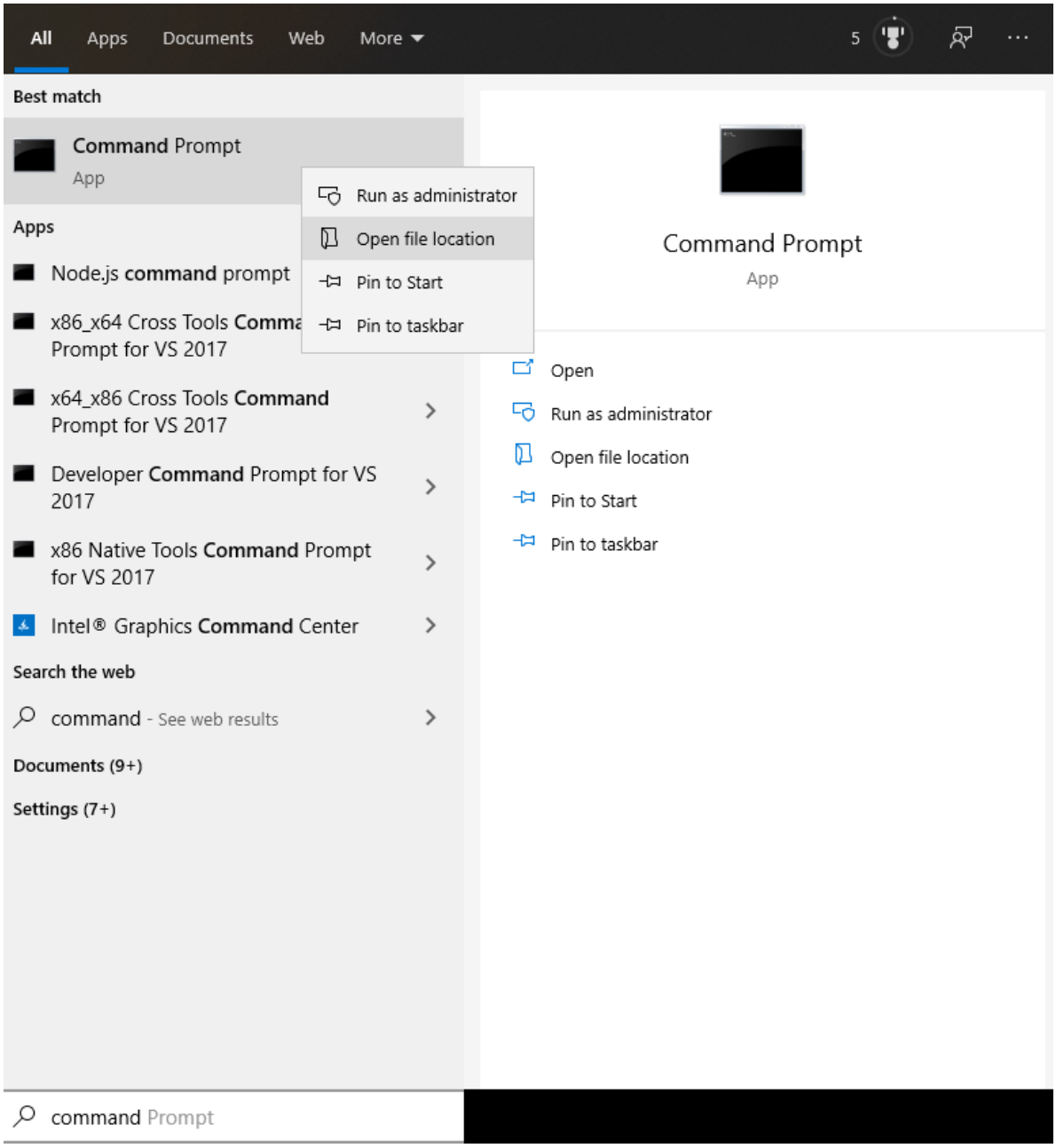
- Search and start Ubuntu, execute the following command

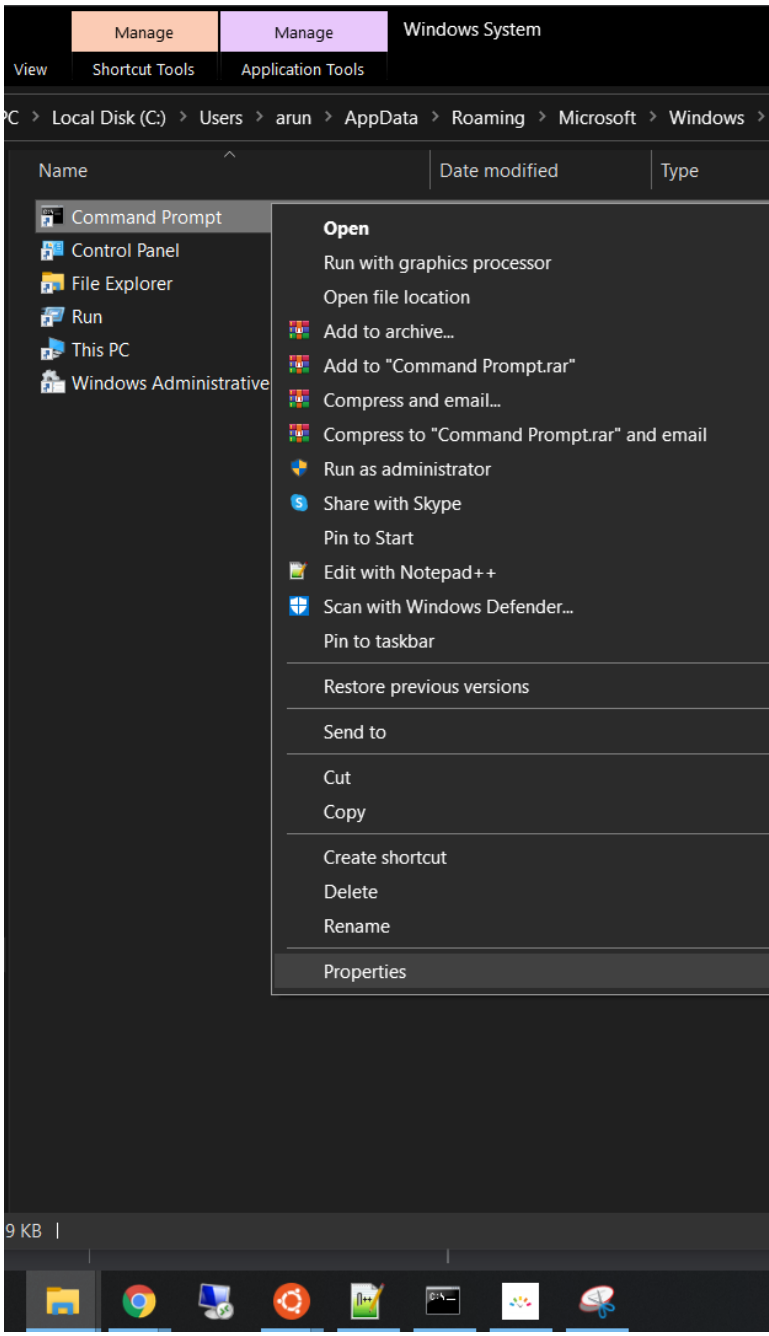
```
sudo ln -s /home/arun/.local/bin/miRge3.0 /usr/local/bin/miRge3.0
sudo ln -s /home/arun/.local/bin/cutadapt /usr/local/bin/cutadapt
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie /usr/local/bin/bowtie
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie-build /usr/local/bin/
↪bowtie-build
sudo ln -s /home/arun/software/bowtie-1.2.3-linux-x86_64/bowtie-inspect /usr/local/
↪bin/bowtie-inspect
```

Change Command Prompt Properties

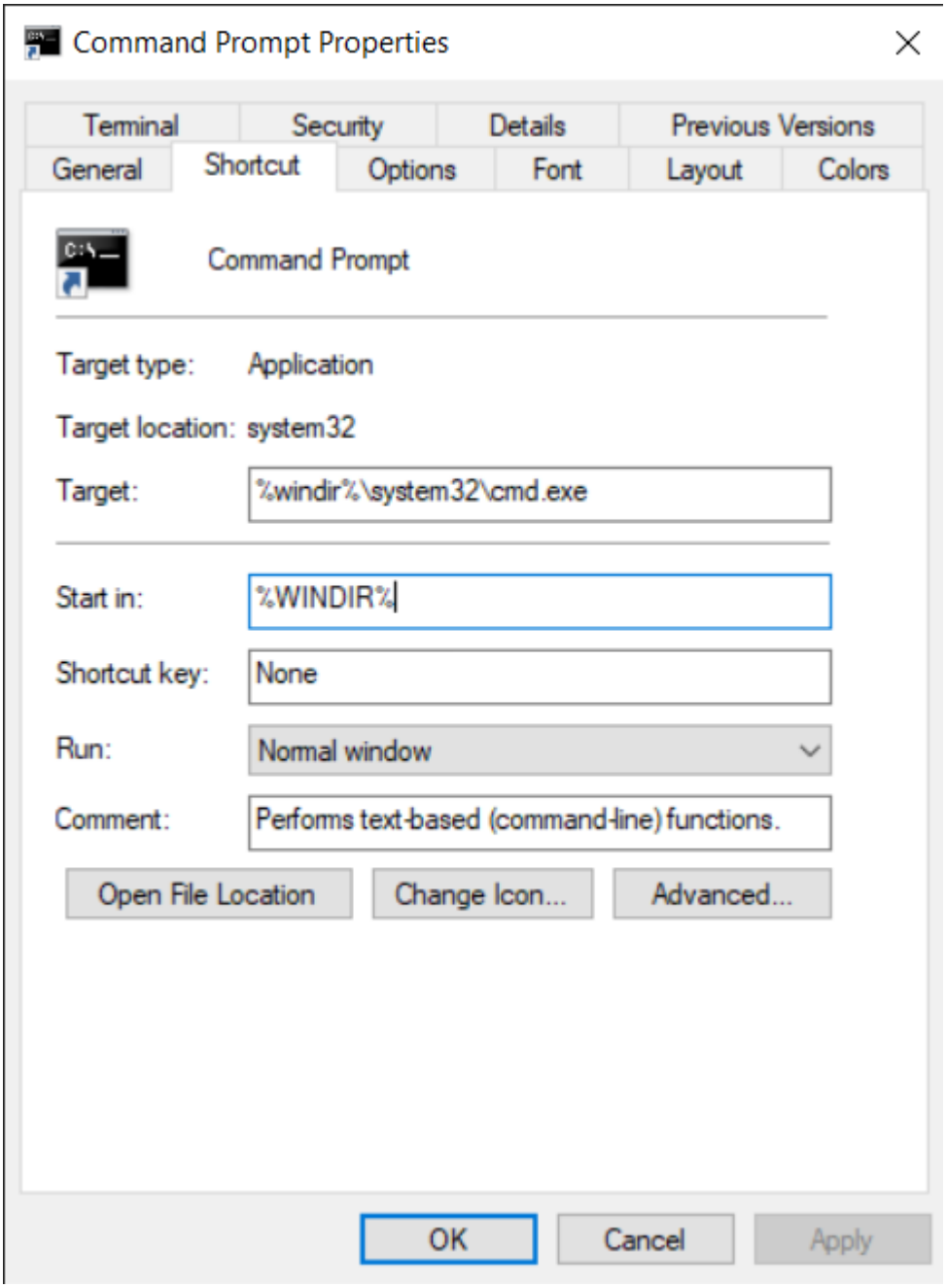
One last thing to avoid an error `The directory name is invalid`:

- Type cmd in Windows search box, right-click on Command Prompt and select Open file location.


All
Apps
Documents
Web
More
5
Best match
Command Prompt
App
Apps
Node.js command prompt
x86_x64 Cross Tools Comma
Prompt for VS 2017
x64_x86 Cross Tools Command
Prompt for VS 2017
Developer Command Prompt for VS
2017
x86 Native Tools Command Prompt
for VS 2017
Intel® Graphics Command Center
Search the web
command - See web results
Documents (9+)
Settings (7+)
Run as administrator
Open file location
Pin to Start
Pin to taskbar
Command Prompt
App
Open
Run as administrator
Open file location
Pin to Start
Pin to taskbar
command Prompt



- Right-click on Command Prompt and click on Properties.
- Under the Shortcut tab, replace `Start in` option by changing the value `%HOMEDRIVE%%HOMEPATH%` to

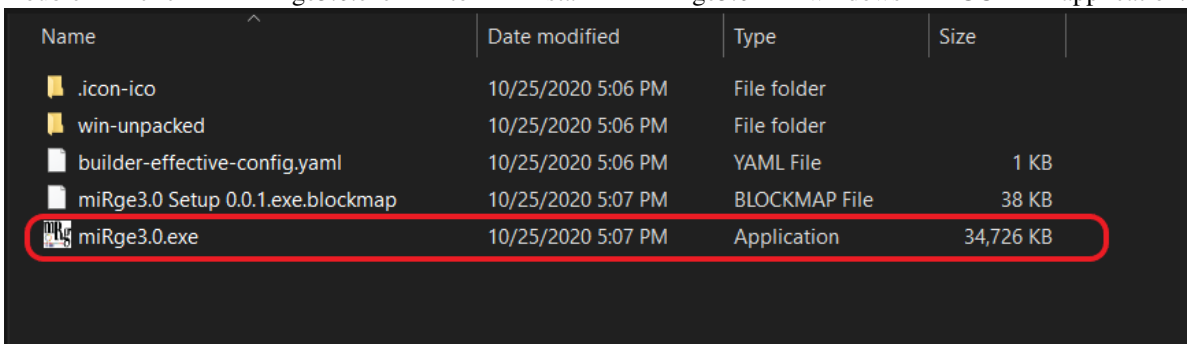


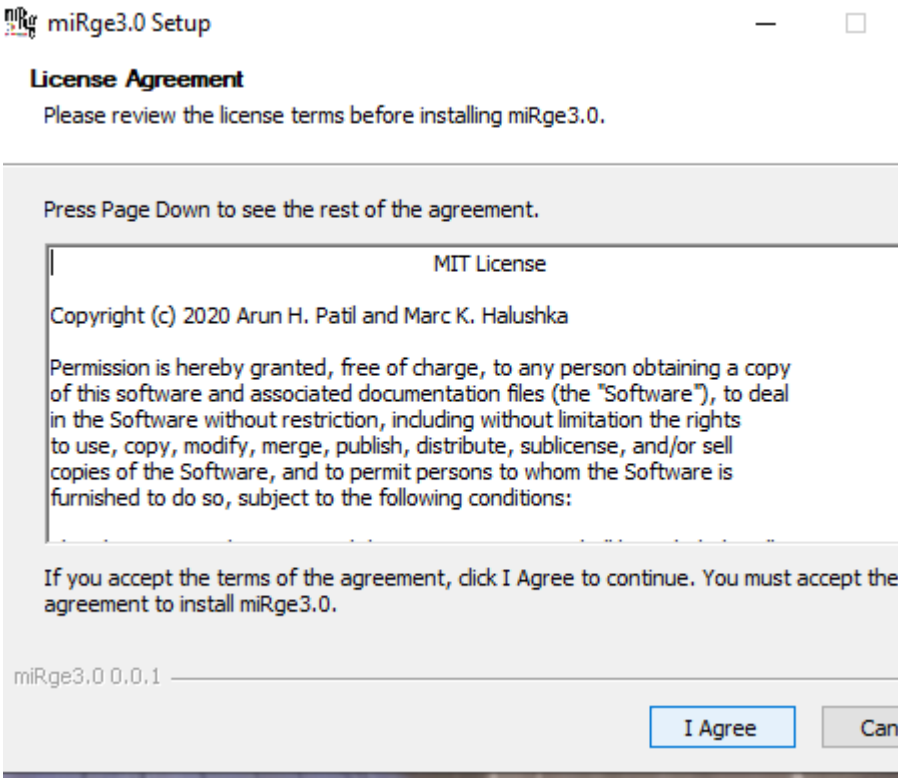
`%WINDIR%`. Click OK.

– Reference 1. Stellarinfo 2. Microsoft

Obtaining and installing GUI application

- Download GUI for Windows 10
- Double click miRge3.0.exe to install miRge3.0 windows GUI application.





- Click Next to complete miRge3.0 installation

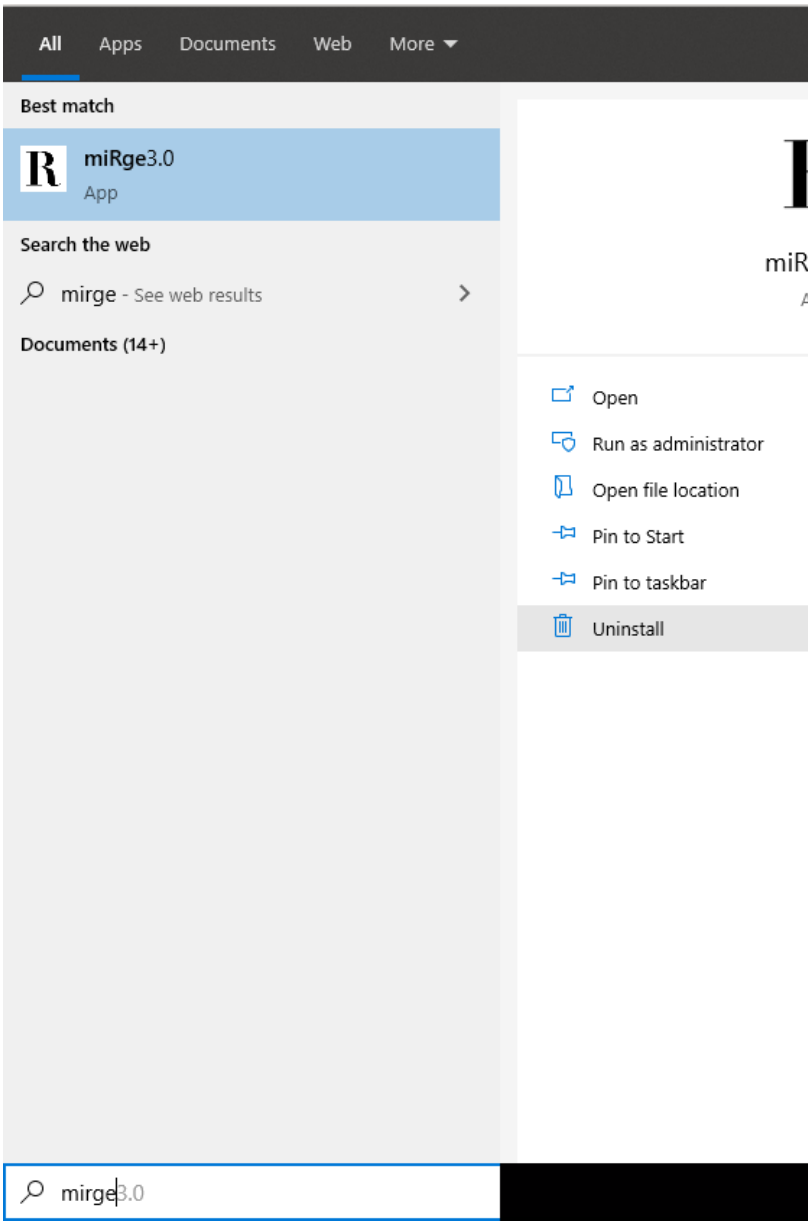
Uninstalling miRge3.0

- Step 1: To uninstall open the terminal and type:

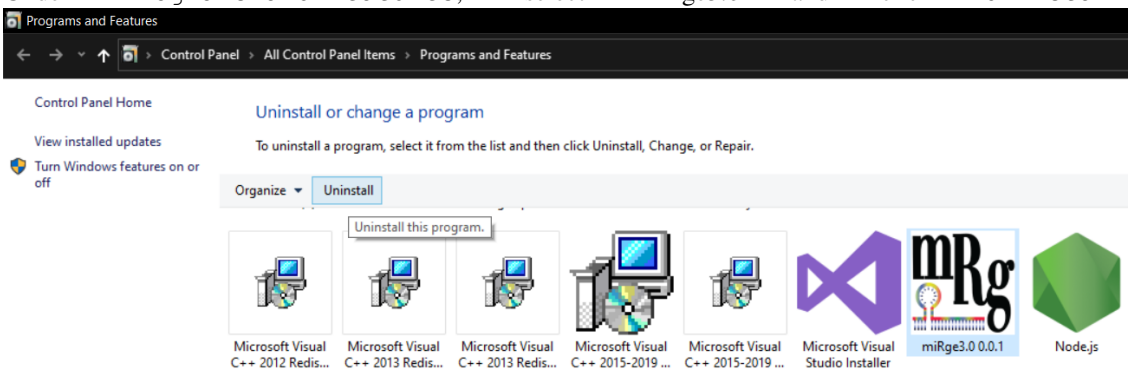
```
python3.8 -m uninstall mirge3
```

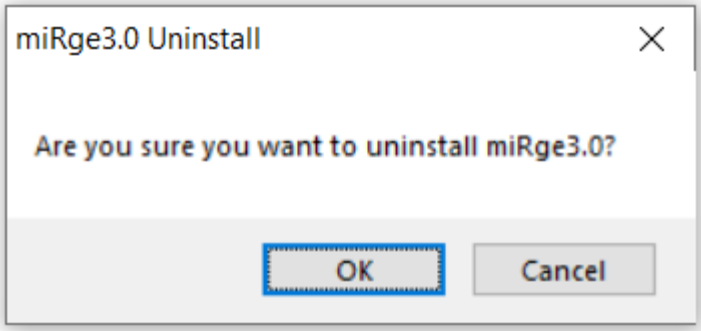
- Step 2:

- Search miRge3.0, right click and select uninstall



- Under `Programs and Features`, select miRge3.0 and click `Uninstall`





– Then select `Uninstall` by clicking `Ok`. Done.

2.2 User guide

2.2.1 Parameters

To view command-line parameters type `miRge3.0 -h`:

```
usage: miRge3.0 [options]

miRge3.0 (Comprehensive analysis of small RNA sequencing Data)

optional arguments:
  -h, --help  show this help message and exit
  --version   show program's version number and exit

Options:
  -s,    --samples            list of one or more samples separated by comma or a␣
↪file with list of samples separated by new line (accepts *.fastq, *.fastq.gz)
  -db,   --mir-DB             the reference database of miRNA. Options: miRBase and␣
↪miRGeneDB (Default: miRBase)
  -lib,  --libraries-path     the path to miRge libraries
  -on,   --organism-name      the organism name can be human, mouse, fruitfly,␣
↪nematode, rat or zebrafish
  -ex,   --crThreshold        the threshold of the proportion of canonical reads for␣
↪the miRNAs to retain. Range for ex (0 - 0.5), (Default: 0.1)
  -phr,  --phred64            phred64 format (Default: 33)
  -spk,  --spikeIn            switch to annotate spike-ins if spike-in bowtie index␣
↪files are located at the path of bowtie's index files (Default: off)
  -ie,   --isoform-entropy    switch to calculate isomir entropy (default: off)
  -cpu,  --threads            the number of processors to use for trimming, qc, and␣
↪alignment (Default: 1)
  -ai,   --AtoI               switch to calculate A to I editing (Default: off)
  -tcf   --tcf-out            switch to write trimmed and collapsed fasta file␣
↪(Default: off)
  -gff   --gff-out            switch to output isomiR results in gff format (Default:␣
↪off)
  -bam   --bam-out            switch to output isomiR results in gff format (Default:␣
↪off)
  -trf   --tRNA-frag          switch to analyze tRNA fragment and halves (Default:␣
↪off)
  -o     --outDir             the directory of the outputs (Default: current␣
↪directory)
```

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```
  -shh    --quiet              enable quiet/silent mode, only show warnings and errors␣
↪(Default: off)

Data pre-processing:
  -a,     --adapter            Sequence of a 3' adapter. The adapter and subsequent␣
↪bases are trimmed
  -g,     --front              Sequence of a 5' adapter. The adapter and any preceding␣
↪bases are trimmed
  -u,     --cut                Remove bases from each read. If LENGTH is positive,␣
↪remove bases from the beginning. If LENGTH is negative, remove bases from the end
  -nxt,   --nextseq-trim       NextSeq-specific quality trimming (each read). Trims␣
↪also dark cycles appearing as high-quality G bases
  -q,     --quality-cutoff     Trim low-quality bases from 5' and/or 3' ends of each␣
↪read before adapter removal. If one value is given, only the 3' end is trimmed
                               If two comma-separated cutoffs are given, the 5' end is␣
↪trimmed with the first cutoff, the 3' end with the second
  -l,     --length             Shorten reads to LENGTH. Positive values remove bases␣
↪at the end while negative ones remove bases at the beginning. This and the following
                               modifications are applied after adapter trimming
  -NX,    --trim-n             Trim N's on ends of reads
  -m,     --minimum-length     Discard reads shorter than LEN. (Default: 16)
  -umi,   --uniq-mol-ids       Removes PCR duplicates and trim UMI of length by␣
↪specifying two comma-separated cutoffs as 5' cutoff,3' bp from both ends of the␣
↪read. eg: 4,4 or 0,4
  -udd,   --umiDedup           Specifies argument to removes PCR duplicates (Default:␣
↪False); if TRUE it will remove UMI and remove PCR duplicates otherwise it only␣
↪remove UMI and keep the raw counts
  -umiq, --umiqiagen           Removes PCR duplicates of reads obtained from Qiagen␣
↪platform (Default: Illumina; "-umi x,y " Required)

Predicting novel miRNAs:
  The predictive model for novel miRNA detection is trained on human and mouse!
  -nmir, --novel-miRNA         include prediction of novel miRNAs
  -minl, --minLength           the minimum length of the reatined reads for novel␣
↪miRNA detection (default: 16)
  -maxl, --maxLength           the maximum length of the reatined reads for novel␣
↪miRNA detection (default: 25)
  -c,     --minReadCounts      the minimum read counts supporting novel miRNA␣
↪detection (default: 2)
  -mloc, --maxMappingLoci      the maximum number of mapping loci for the retained␣
↪reads for novel miRNA detection (default: 3)
  -sl,    --seedLength         the seed length when invoking Bowtie for novel miRNA␣
↪detection (default: 25)
  -olc,   --overlapLenCutoff   the length of overlapped seqence when joining reads␣
↪into longer sequences based on the coordinate
                               on the genome for novel miRNA detection (default: 14)
  -clc,   --clusterLength      the maximum length of the clustered sequences for novel␣
↪miRNA detection (default: 30)

Optional PATH arguments:
  -pbwt, --bowtie-path         the path to system's directory containing bowtie binary
  -psam, --samtools-path       the path to system's directory containing samtools␣
↪binary
  -prf,   --RNAfold-path       the path to system's directory containing RNAfold binary
```

2.2.2 miRge3.0 libraries

miRge3.0 pipeline aligns the raw reads against a set of small-RNA annotation libraries. The libraries specific to the organism of interest can be obtained from SourceForge. Downloading the libraries on terminal:

Command-line Interface (CLI)

We recommend to create a directory `miRge3_Lib` and download using wget as shown below,

```
mkdir miRge3_Lib
cd miRge3_Lib
wget -O human.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/human.
↪tar.gz/download"
wget -O mouse.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/mouse.
↪tar.gz/download"
wget -O rat.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/rat.tar.
↪gz/download"
wget -O nematode.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/
↪nematode.tar.gz/download"
wget -O fruitfly.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/
↪fruitfly.tar.gz/download"
wget -O zebrafish.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/
↪zebrafish.tar.gz/download"
wget -O hamster.tar.gz "https://sourceforge.net/projects/mirge3/files/miRge3_Lib/
↪hamster.tar.gz/download"
```

Users can download only what is necessary. Unzip the files once downloaded by the following command:

```
tar -xzf human.tar.gz
```

Replace `human` with the organism of interest. If you want to extract all the files at once, you could use `tar -xzf *.tar.gz` instead.

Graphical User Interface (GUI)

We recommend to create a folder `miRge3_Lib` and download the libraries directly from SourceForge. Once downloaded, extract/unzip the compressed files.

Building new libraries

If you are interested in creating specific library for an organism that is not part of this set then please refer to miRge3_build.

2.2.3 CLI - Example usage

Example command usage:

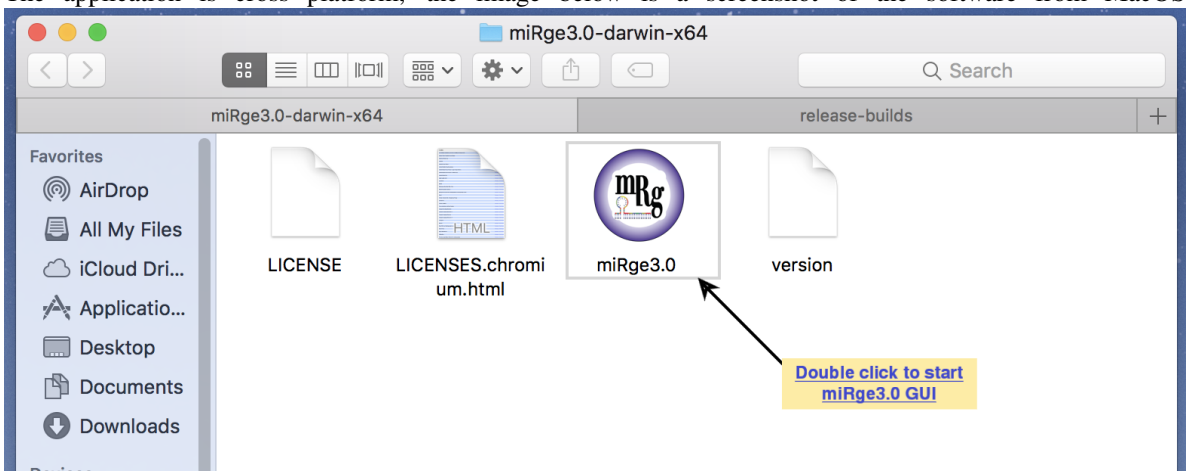
```
miRge3.0 -s SRR772403.fastq,SRR772404.fastq,SRR772405.fastq,SRR772406.fastq -lib␣
↪miRge3_Lib -on human -db mirgenedb -o output_dir -gff -nmir -trf -ai -cpu 12 -a␣
↪illumina
```

Output command line:

```
bowtie version: 1.2.3
Samtools version: 1.7
RNAfold version: 2.4.14
Collecting and validating input files...

miRge3.0 will process 4 out of 4 input file(s).

Cutadapt finished for file SRR772403 in 2.5358 second(s)
Collapsing finished for file SRR772403 in 0.0126 second(s)

Cutadapt finished for file SRR772404 in 7.3542 second(s)
Collapsing finished for file SRR772404 in 0.2786 second(s)

Cutadapt finished for file SRR772405 in 11.0667 second(s)
Collapsing finished for file SRR772405 in 0.8585 second(s)

Cutadapt finished for file SRR772406 in 3.5771 second(s)
Collapsing finished for file SRR772406 in 0.8677 second(s)

Matrix creation finished in 0.3838 second(s)

Data pre-processing completed in 27.2443 second(s)

Alignment in progress ...
Alignment completed in 15.8305 second(s)

Summarizing and tabulating results...
The number of A-to-I editing sites for is less than 10 so that no heatmap is drawn.
Summary completed in 71.4691 second(s)

Predicting novel miRNAs


Performing prediction of novel miRNAs...
Start to predict
Prediction of novel miRNAs Completed (104.83 sec)

The analysis completed in 222.2487 second(s)
```

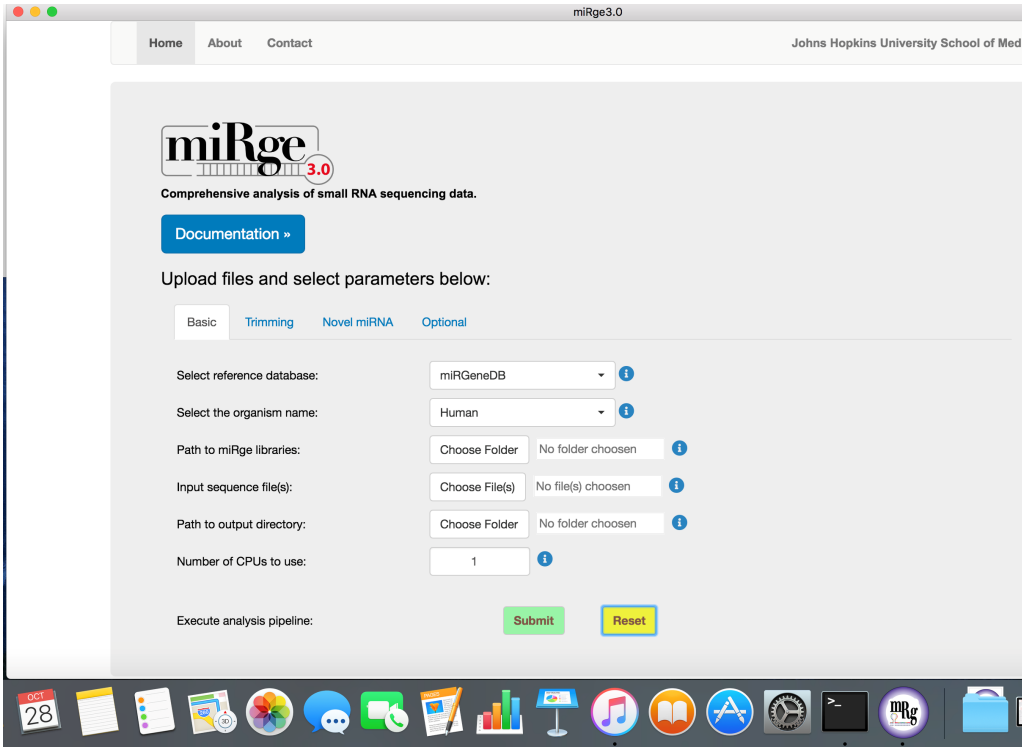
2.2.4 miRge3.0 GUI

- The application is cross platform, the image below is a screenshot of the software from MacOS

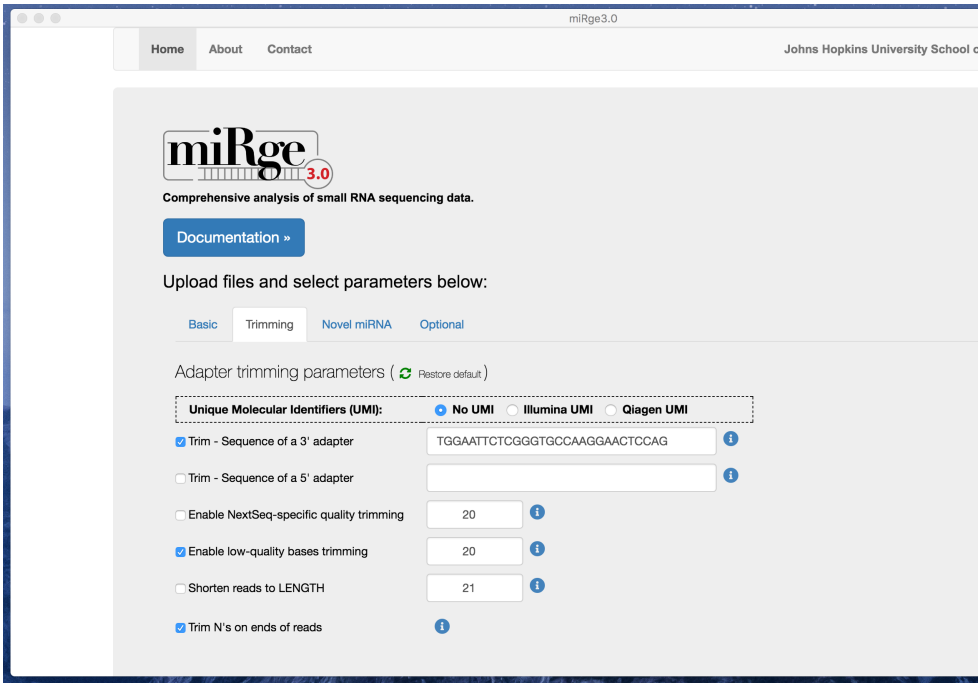


- The software is easy to use with default parameters. The parameters are tabulated into four groups such as basic, trimming parameters, novel miRNA prediction and other optional parameters.

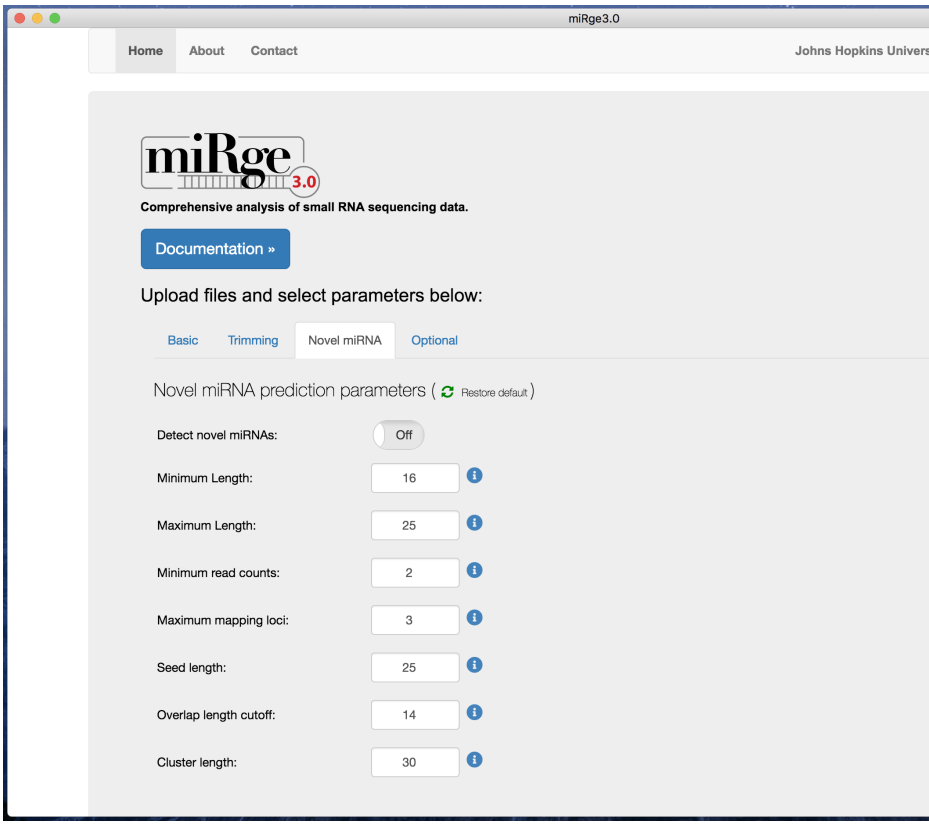
- Screenshot with basic parameters

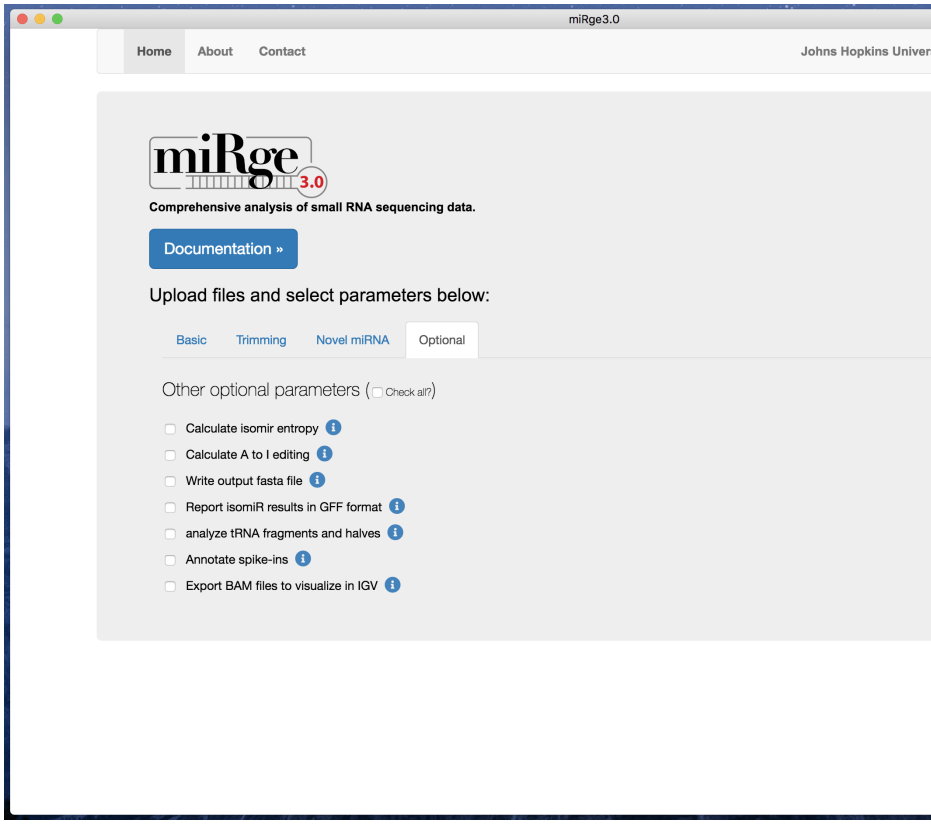


- Screenshot with trimming parameters



- Screenshot with novel miRNA predictions





- Screenshot with other optional parameters

2.2.5 Resources

- Lu, Y., et al., **miRge 2.0 for comprehensive analysis of microRNA sequencing data**. 2018. *BMC Bioinformatics*. PMID.
- Baras, S. A., et al., **miRge - A Multiplexed Method of Processing Small RNA-Seq Data to Determine MicroRNA Entropy**. 2015. *PLoS One*. PMID.

2.3 miRge3.0 output

2.3.1 Command and sample run with UMI datasets

```
miRge3.0 -s SRR8557389.fastq,SRR8557396.fastq,SRR8557398.fastq,SRR8557399.fastq -lib␣
↪miRge3_Lib -on human -db miRGeneDB \
        -o temp -a AACTGTAGGCACCATCAAT -udd --qiagenumi -umi 0,12 -cpu 12 -q 20 -NX -
↪nmir -minl 16 -maxl 25 -c 2 \
        -mloc 3 -sl 25 -olc 14 -clc 30 -gff

bowtie version: 1.2.3
cutadapt version: 2.7
Samtools version: 1.7
RNAfold version: 2.4.14
Collecting and validating input files...

miRge3.0 will process 4 out of 4 input file(s).
```

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```
Cutadapt finished for file SRR8557389 in 21.0854 second(s)
Collapsing finished for file SRR8557389 in 0.0699 second(s)
Cutadapt finished for file SRR8557396 in 10.305 second(s)
Collapsing finished for file SRR8557396 in 0.6016 second(s)
Cutadapt finished for file SRR8557398 in 10.891 second(s)
Collapsing finished for file SRR8557398 in 0.911 second(s)
Cutadapt finished for file SRR8557399 in 14.2126 second(s)
Collapsing finished for file SRR8557399 in 1.1292 second(s)
Matrix creation finished in 0.4788 second(s)

Data pre-processing completed in 62.762 second(s)

Alignment in progress ...
Alignment completed in 16.9863 second(s)

Summarizing and tabulating results...
Summary completed in 7.8131 second(s)
Predicting novel miRNAs

Performing prediction of novel miRNAs...Start to predictPrediction of novel miRNAs␣
↪Completed (220.35 sec)
The analysis completed in 310.7281 second(s)
```

2.3.2 Output tree structure

An output directory is created for each run such as `miRge.2020-10-9_1-35-53`, where the name is followed by date time format `miRge.yy-dd-mm-hr-mm-ss`.

```
The following output is in general, however, the resultant output files are based on␣
↪the options selected during miRge3.0 execution.

miRge.2020-10-9_1-35-53
├── run.log (Gives the detailed log of miRge3.0 execution)
├── unmapped.log (Gives the detailed log of novel miRNA prediction)
├── mapped.csv (CSV file with read counts across each smallRNA library)
├── unmapped.csv (CSV file with unaligned/mapped reads)
├── annotation.report.csv (Basic annotation report with small RNA distribution in CSV␣
↪format)
├── annotation.report.html (Basic annotation report with small RNA distribution in␣
↪HTML format)
├── sample_miRge3.gff (GFF file with reads with isomiRs across one or more samples,␣
↪if -gff option selected)
├── miR.Counts.csv (miRNA raw read counts across samples)
├── miR.RPM.csv (miRNA Read Per Million - RPM counts across samples)
├── *_umiCounts.csv (Counts for each unique UMI for each sample)
├── index_data.js (Javascript file with data generated for visualization)
├── miRge3_visualization.html (HTML for data visualization)
├── FOLDER_novel_miRNAs
│   ├── *.pdf (novel miRNA structure in PDF format for each miRNA)
│   └── sample_novel_miRNAs_report.csv (Contains list of identified novel miRNAs in␣
↪CSV format)
├── a2IEditing.detail.txt
├── a2IEditing.report.csv
├── a2IEditing.report.newform.csv
```

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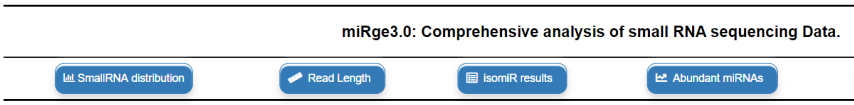
```
├── tRFs.potential.report.tsv
├── tRF.Counts.csv
├── tRF.RP100K.csv
├── tRFs.potential.report.tsv
├── discarded.reads.summary.assigningtRFs.csv
└── tRFs.samples.tmp
    └── *.tRFs.* (Detailed summary of tRFs from each sample)
```

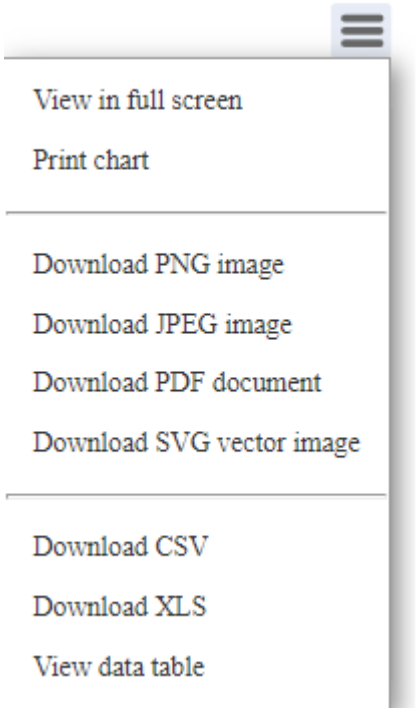
2.3.3 miRge - interactive visualization

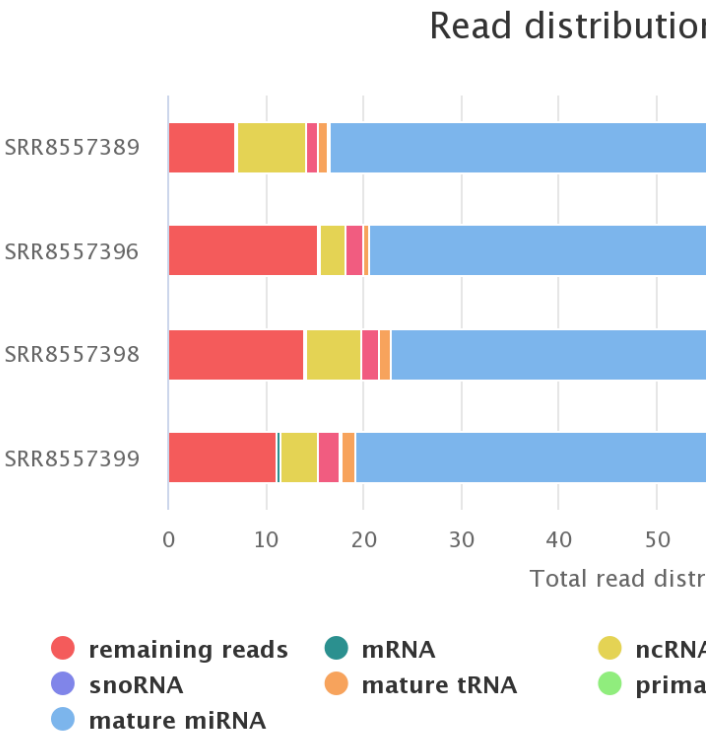
miRge3.0 produces several interactive visualization graphics as follows

- Screenshot of the miRge visualization HTML tab

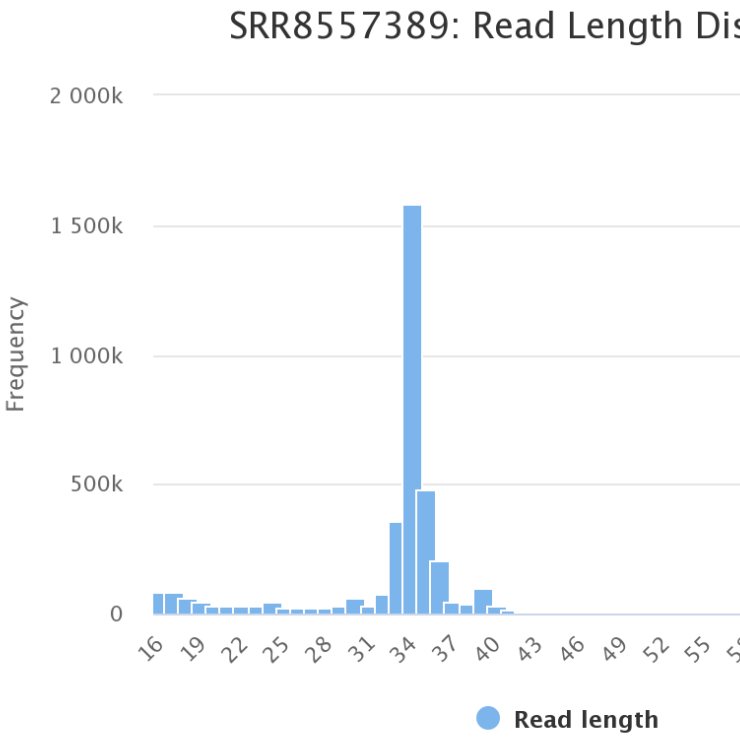


- Chart view and download options

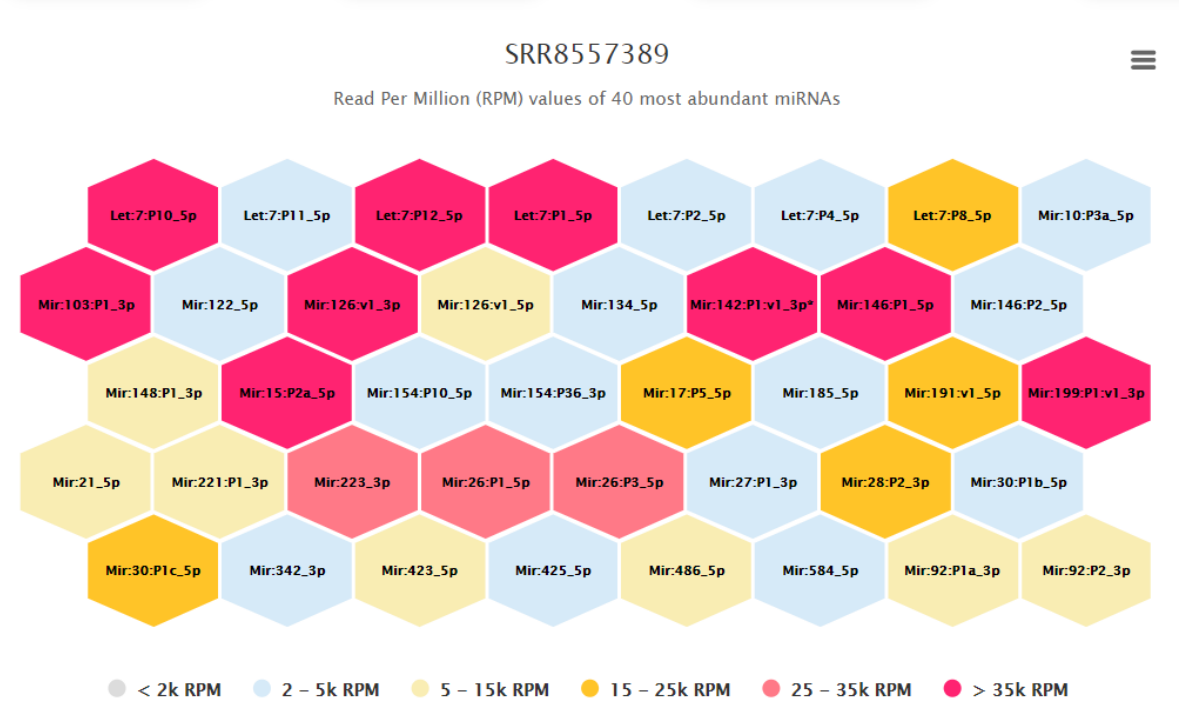




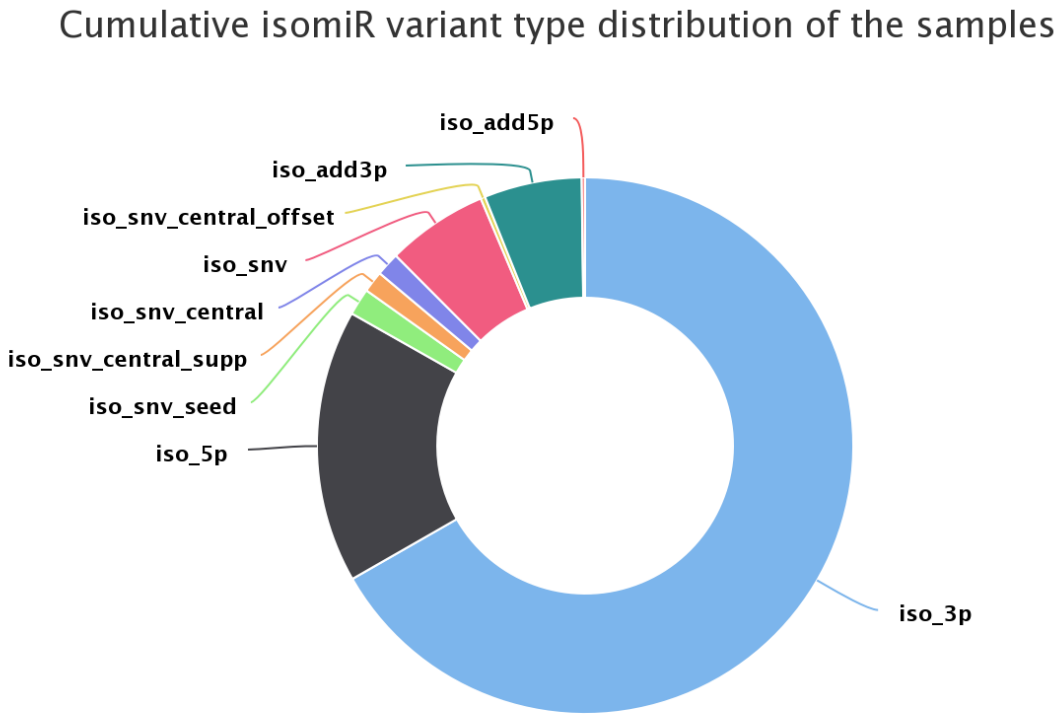
- Screenshot of the smallRNA read distribution for each sample



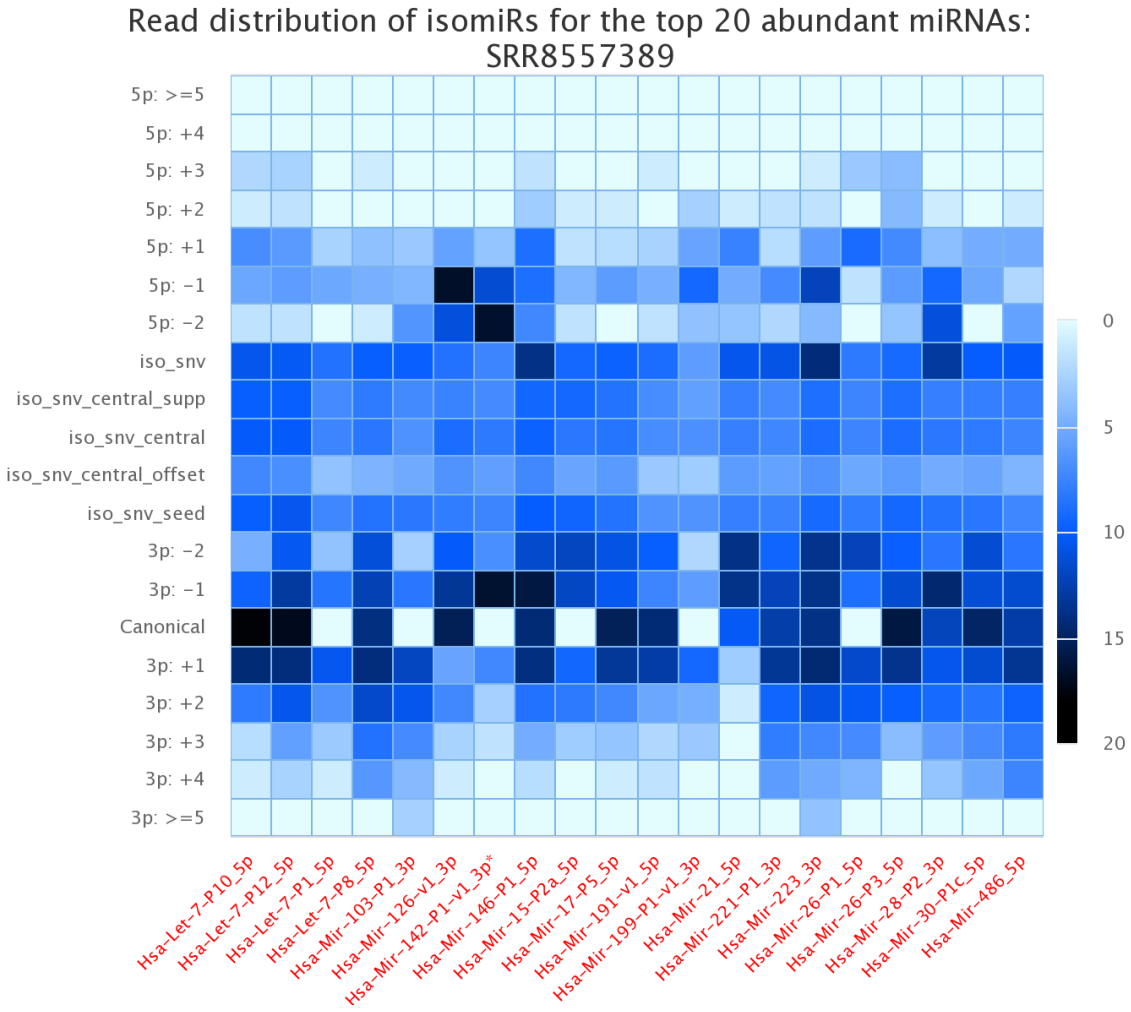
- Screenshot of the read length distribution for each sample
- Screenshot of the tile map representing top 40 high abundant miRNAs for each study



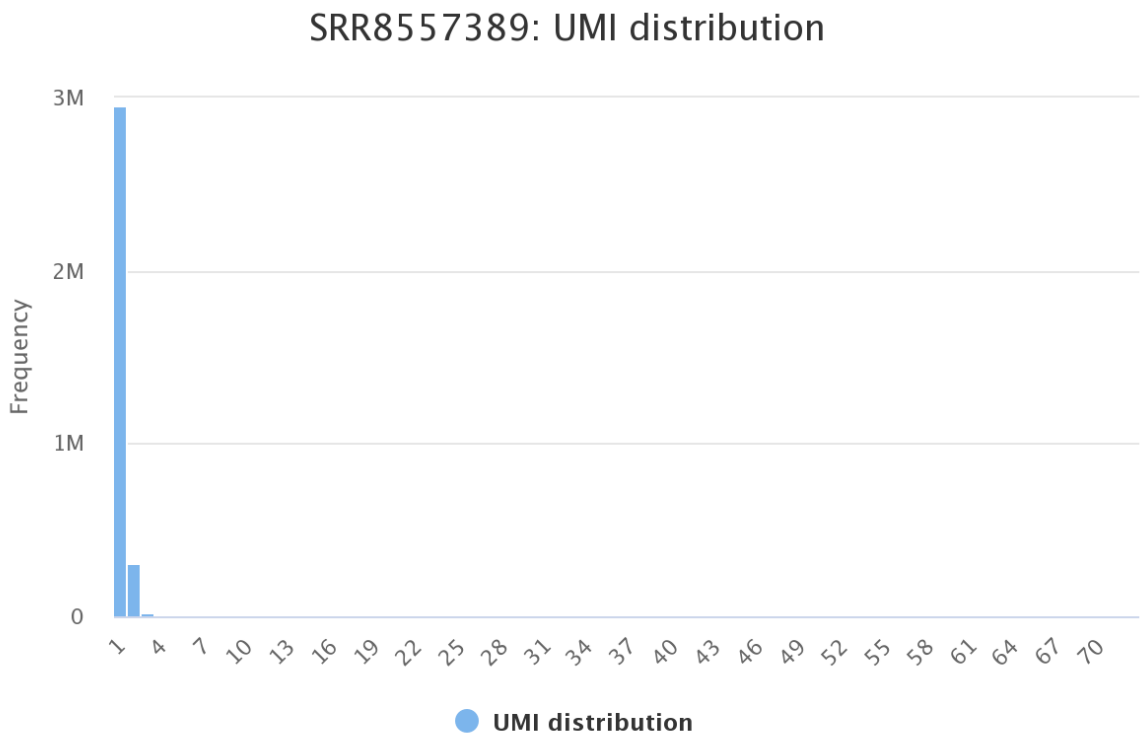
- Screenshot of the variant distribution for all samples combined (isomiRs)



- Screenshot of the heatmap representing variants for each sample for the top 20 high abundant miRNAs (isomiRs)



- Screenshot of the histogram representing UMI counts across each sample



Show 10 entries Search:

id	Name	Probability	Chr	Start pos.	End Pos
1	SRR8557389_novel_miRNA_1	0.9999987516742271	chr2	134127130	134127
2	SRR8557389_novel_miRNA_2	0.9999915571402658	chr1	172138858	172138
3	SRR8557389_novel_miRNA_3	0.999982270847492	chr3	113594918	113594
4	SRR8557389_novel_miRNA_4	0.9963371234941661	chr8	27433413	274334
5	SRR8557389_novel_miRNA_5	0.9938801463496213	chr17	81125886	811259
6	SRR8557389_novel_miRNA_6	0.9908373862619209	chr17	74748663	747486
7	SRR8557389_novel_miRNA_7	0.9905492996218249	chr12	69584745	695847
8	SRR8557389_novel_miRNA_8	0.9781204295092701	chr22	20086072	200860
9	SRR8557389_novel_miRNA_9	0.9723790135555249	chr5	141849784	141849
10	SRR8557389_novel_miRNA_10	0.9655899863443722	chr19	13836292	138363

Showing 1 to 10 of 26 entries Previous 1

- Screenshot of a list of novel miRNAs identified across samples

2.3.4 Resources:

The graphics for miRge3.0 visualization is enabled with javascripts and CSS obtained from the following:

- Interactive charts from HighCharts
- Icons from Font Awesome
- Interactive HTML table

2.4 MIT License