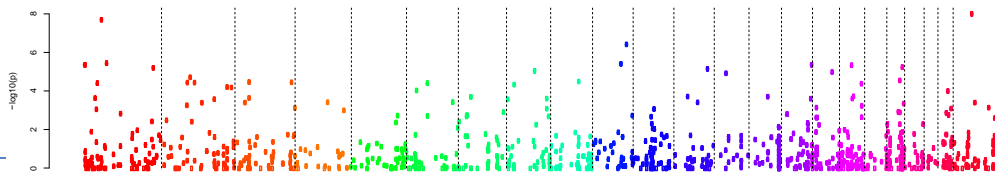


This tutorial explains in five steps how miFrame can be applied to perform the analysis of miRNA NGS data.



And the different output formats and graphics

main homepage with introduction, information such as the tutorial and example data and the three input options

miFrame

About

While in the past decades nucleic acid analysis has been predominantly carried out using quantitative low- and high-throughput approaches such as qRT-PCR and microarray technology, NGS with its single base resolution is now frequently applied in DNA and RNA testing. Especially for small non-coding RNAs such as microRNAs there is a need for analysis and visualization tools that facilitate interpretation of the results also for clinicians. We developed miFrame, which supports various standard input formats from NGS data. Our tool carries out quantitative and qualitative analyses for known as well as predicted novel microRNAs and presents the results in a well interpretable manner. Analyses include among others expression analysis of precursors and mature miRNAs, detection of novel precursors and detection of potential iso-microRNAs. Aggregation of results from different users moreover allows for evaluation whether remarkable results, such as novel mature miRNAs, are indeed specific for the respective experimental set-up or are frequently detected across a broad range of experiments.

There are three options available for input. The first option accesses a previously calculated case via case id and password. If you visit the web site for the first time you start with option 2 or option 3. Option 2 is mainly the input via mirdeep2 generated arf data. There are examples files how these input files should look like. Option 3 is mainly basecounts input. Here are also example files on how these basecounts files should look like.

- Information ▼
- Option 1: Case ID ▼
- Option 2: mirdeep2 input ▼
- Option 3: basecounts input ▼

Input Option 1: Users can provide a case id and a password. If a previous analysis with respective ID and password has been carried out, the pre-computed results can be accessed. Data are available for 12 months.

Information ▼

Option 1: Case ID ▲

Option 1: Description ?

In this option you can enter already computed cases, which were calculated with option 2 and option 3. When using option 2 or option 3, you get a case id and a password to re-enter the cases.

Option 1: Enter Case ID ?

Case ID
ba87ad68b2

Password

Enter

Option 2: mirdeep2 input ▼

Option 3: basecounts input ▼

TTCAAAATTTCACAAAAAGAT
GTGATTACATACAAATCGGACAT
TTTGTCACTACATTTGCAAGTAT
GTAAGTTGATGAGACAGAAAATGT

Input Option 2: Here, the user can upload files pre-processed with miRDeep (arf format). In addition to the arf files, a read count file has to be provided that contains for each arf file the number of reads for normalization purpose. Finally, a file containing the class labels has to be provided.

Information ▼

Option 1: Case ID ▼

Option 2: mirdeep2 input ▲

Option 2: Examples ?

Example case input:

mirdeep2 arf zip file: [example_arf_files.zip](#) (arf is the output of the mirdeep2 tool)

total counts file: [example_total_counts.txt](#) (number of reads per sample)

id folder file: [example_id_folder.csv](#) (samples are separated into groups. group name for each sample is in tab separated lines)

Show pre-computed example case

Option 2: Select input files ?

Upload mirdeep2 arf zip file:
Durchsuchen... Keine Datei ausgewählt.

Upload total counts file:
Durchsuchen... Keine Datei ausgewählt.

Upload id folder file:
Durchsuchen... Keine Datei ausgewählt.

Upload files

Option 3: basecounts input ▼

Input Option 3: In the third case users can provide miRNA mapping files that have been generated by other software packages and processed by the user. miFrame expects per miRNA one file, the first row corresponding to the miRNA sequence and then for each sample an won row with the read counts mapping to that position

The screenshot displays the miFrame web application interface. At the top, a sidebar menu on the left contains the following options: 'Information', 'Option 1: Case ID', 'Option 2: mirdeep2 input', and 'Option 3: basecounts input'. The 'Option 3: basecounts input' option is currently selected and highlighted. To the right of the sidebar, a panel titled 'Option 3: Examples' provides instructions for using the basecounts input. It states: 'Example case input: basecounts zip file: [example_basecounts.zip](#) (base counts per miRNA. For each miRNA one file.)' and 'id folder file: [example_id_folder.csv](#) (samples are separated into groups. group name for each sample is in tab separated lines)'. Below this text is a button labeled 'Show pre-computed example case'. Further down, another panel titled 'Option 3: Select input files' contains two file upload sections. The first section is for the 'basecounts zip file' and the second is for the 'id folder file'. Each section includes a 'Durchsuchen...' (Browse...) button and the text 'Keine Datei ausgewählt.' (No file selected.). At the bottom of this panel is a button labeled 'Upload files'.

Information

Option 1: Case ID

Option 2: mirdeep2 input

Option 3: basecounts input

Option 3: Examples ?

Example case input:

basecounts zip file: [example_basecounts.zip](#) (base counts per miRNA. For each miRNA one file.)

id folder file: [example_id_folder.csv](#) (samples are separated into groups. group name for each sample is in tab separated lines)

Show pre-computed example case

Option 3: Select input files ?

Upload basecounts zip file:

Durchsuchen... Keine Datei ausgewählt.

Upload id folder file:

Durchsuchen... Keine Datei ausgewählt.

Upload files

In the second step, miFrame presents an overview of the input, e.g. whether data upload has been done successfully. Our tool also outputs the number of miRNAs in the study (in the case below 1,322 different miRNAs)

Then the user can select from all groups that were annotated in the ID file which two groups should be compared to each other.

File miframe_input_brain_normalized.zip upload OK.
File id_folder_1.txt upload OK.

basecounts number is : 1322

Step 2/5:

1 2 3 4 5

TTTGCTAAACAA
AAATAATAGAAA
TATTACTTAACAA
AATAAAGATATCT

ACGGTTT
GTATTGACGGG
TATTGTTCCCA

AAAAATTGT
GATTACAIA
GTCAIACTA
AGTTGATC

CCTAAAGAA
TAATAGAAA
TACTTAACA
AAAGATATC

Select group names?

Group 1	Group 2
AD <input type="radio"/>	AD <input type="radio"/>
AD_control <input type="radio"/>	AD_control <input type="radio"/>
MS_control <input type="radio"/>	MS_control <input checked="" type="radio"/>
MCI <input type="radio"/>	MCI <input type="radio"/>
MS <input checked="" type="radio"/>	MS <input type="radio"/>

Proceed

In the third step, Users can specify the input parameters, the significance level (a typical value is 0.05), the number of base positions on the precursor that have to be below the alpha level and the window size.

Step 3/5:

1 2 3 4 5

Select parameter ?

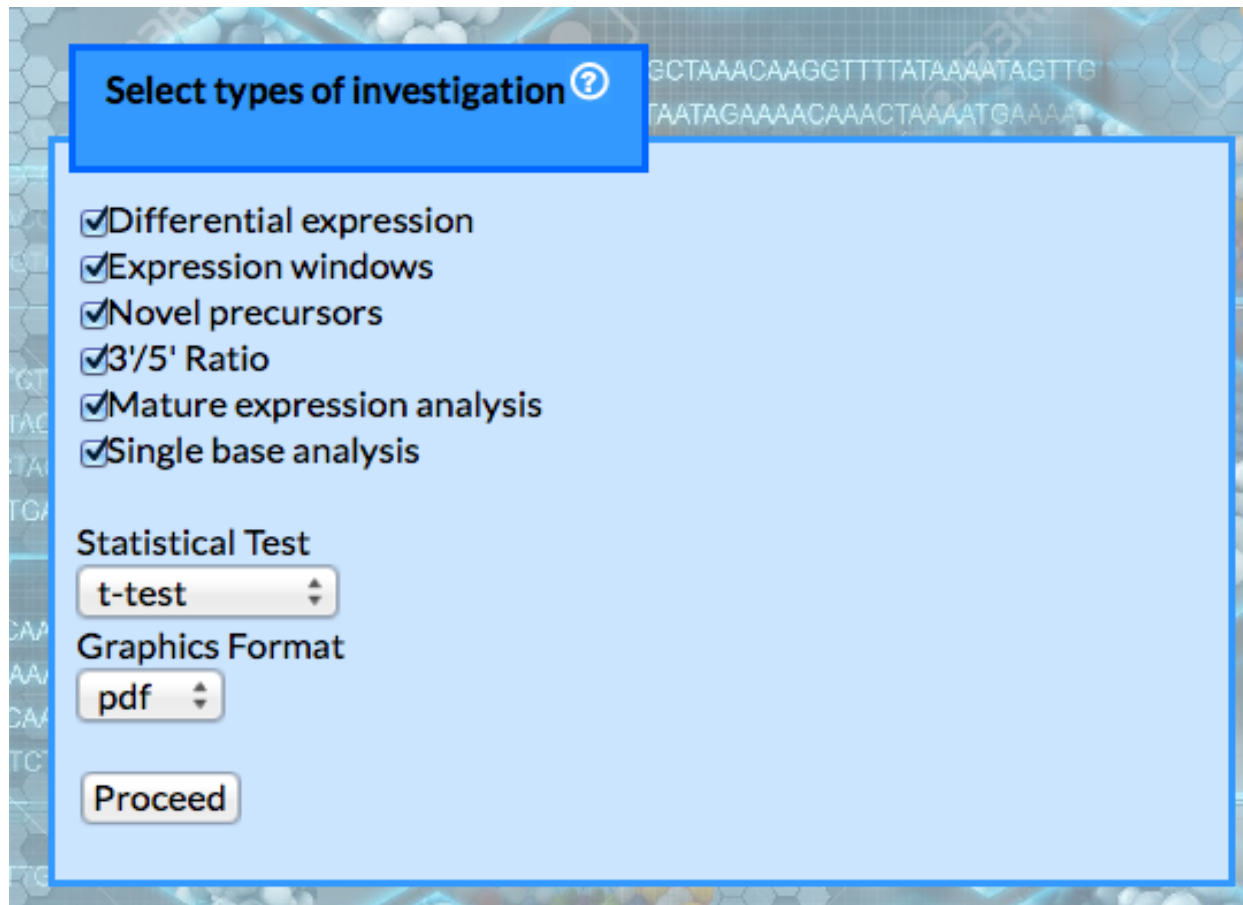
Requested significance threshold
0.05

Requested number of sig base threshold
15

Requested window for rat. cod. miRNA
2

Proceed

In the fourth step, the analyses to be performed can be selected, whether parametric t-test or non-parametric WMW test should be carried out and which graphical output should be used.



The screenshot shows a software window titled "Select types of investigation" with a help icon. The window has a light blue background and a blue border. It contains a list of six checked options: "Differential expression", "Expression windows", "Novel precursors", "3'/5' Ratio", "Mature expression analysis", and "Single base analysis". Below this list are two dropdown menus: "Statistical Test" set to "t-test" and "Graphics Format" set to "pdf". At the bottom is a "Proceed" button. The background of the window features a faint DNA sequence and a molecular structure.

Select types of investigation ?

- ☒ Differential expression
- ☒ Expression windows
- ☒ Novel precursors
- ☒ 3'/5' Ratio
- ☒ Mature expression analysis
- ☒ Single base analysis

Statistical Test
t-test

Graphics Format
pdf

Proceed

Finally, users have to approve that miFrame is non-commercial and can select whether their data should be used for the aggregation analysis.

Step 5/5:

1 2 3 4 5

Calculate your input ?

☒ The use of miRTrail is free for non-commercial use.

☒ aggregation of results: the relevant miRNAs from your analysis will be stored anonymously. In exchange you will get aggregated results of all analyses. From that, you can understand in how many percent of all analyses your miRNAs were relevant.

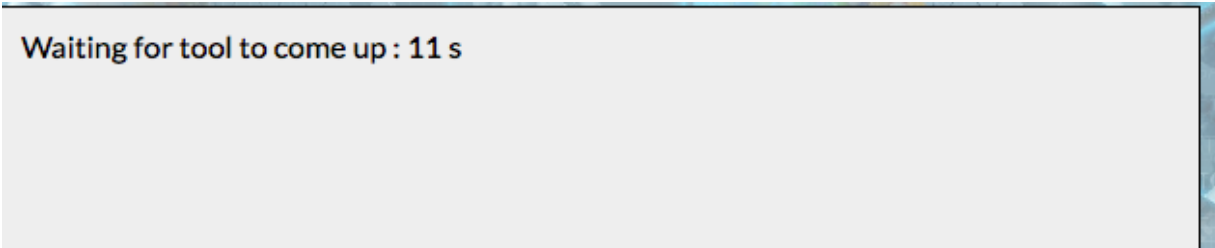
Calculate

During the processing, status updates such as the run time and which files are currently processed are provided to the user. The examples that are presented in the manuscript and are available for download as examples required around 10 minutes computing time.

Waiting for tool to come up : 11 s

Processing basecounts_hsa-mir-1302-1.txt.
Processing basecounts_hsa-mir-1302-10.txt.
Processing basecounts_hsa-mir-1302-11.txt.
Processing basecounts_hsa-mir-1302-2.txt.
Processing basecounts_hsa-mir-1302-3.txt.
Processing basecounts_hsa-mir-1302-4.txt.
Processing basecounts_hsa-mir-1302-5.txt.
Processing basecounts_hsa-mir-1302-6.txt.

During the processing, status updates such as the run time and which files are currently processed are provided to the user. The examples that are presented in the manuscript and are available for download as examples required around 10 minutes computing time.

A screenshot of a software interface with a light gray background. In the top-left corner, the text "Waiting for tool to come up : 11 s" is displayed in a dark font. The rest of the interface is mostly blank, with a vertical blue and white pattern visible on the right edge.

Waiting for tool to come up : 11 s

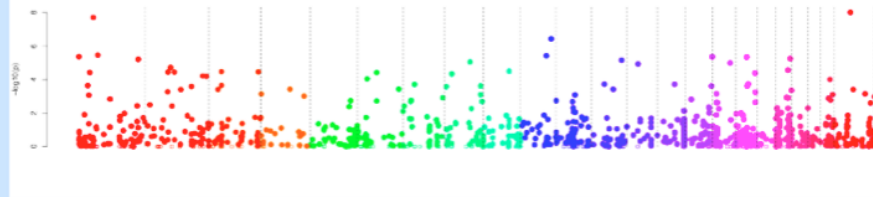
As output an overview page is generated. From this page the different analysis results can be accessed

miFrame

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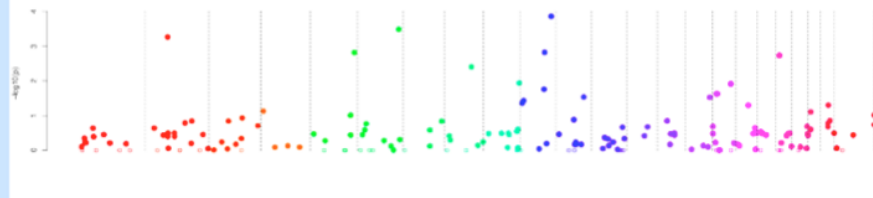
Download results as a [file](#).

expression analysis



[Show details](#)

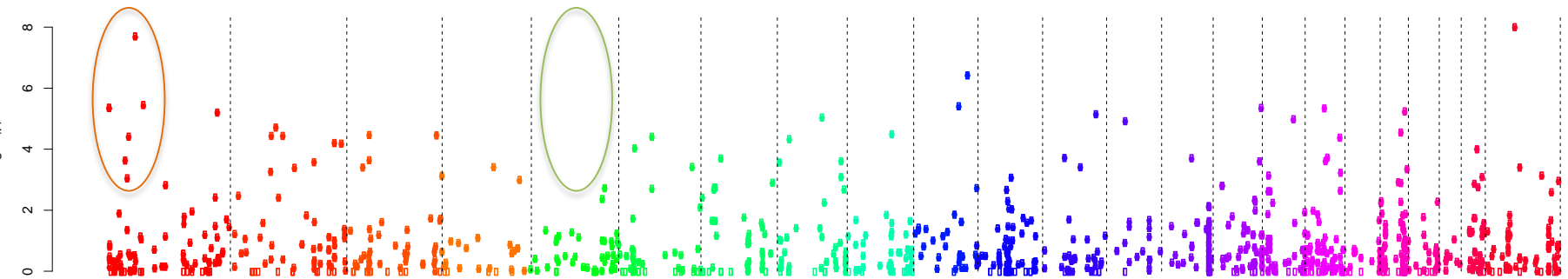
novelprecursor analysis



[Show details](#)

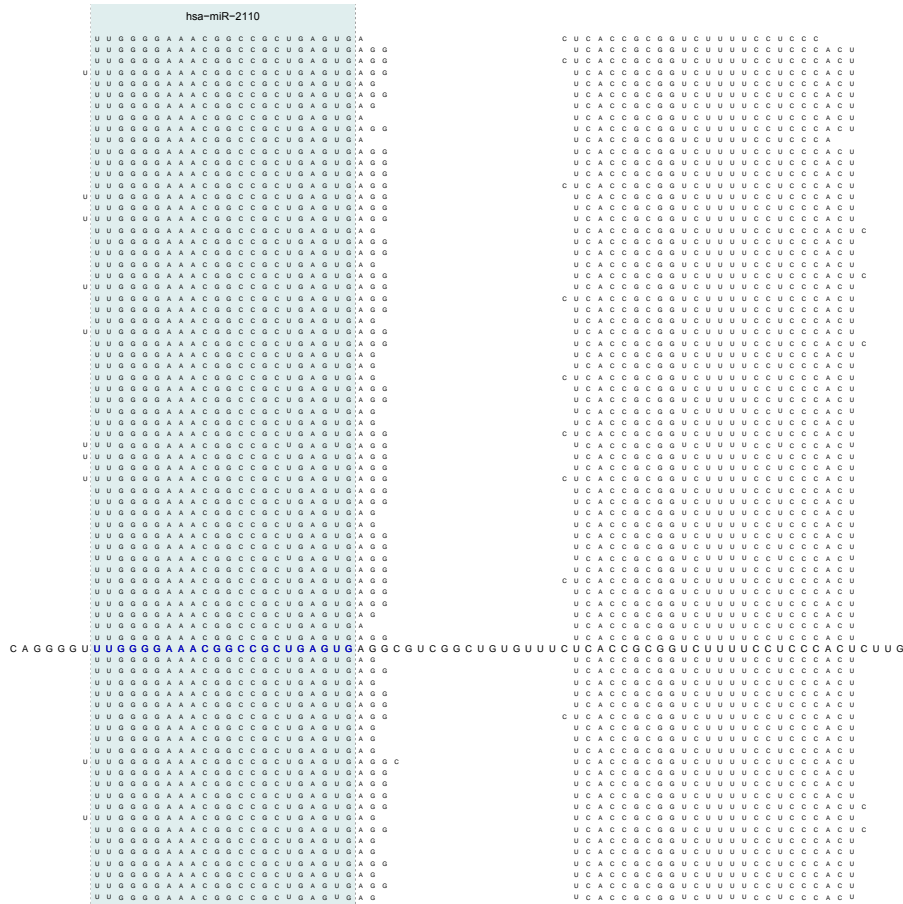
First, manhattan plots are presented for each analysis. These show the negative decimal logarithm of p-values for each miRNA ordered with respect to the chromosomal position. Clusters of high dots on a certain chromosome mean that in this genomic region significant results accumulate.

The orange part denotes such a region while the green region does not contain significant results



miFrame presents three general types of results graphics.

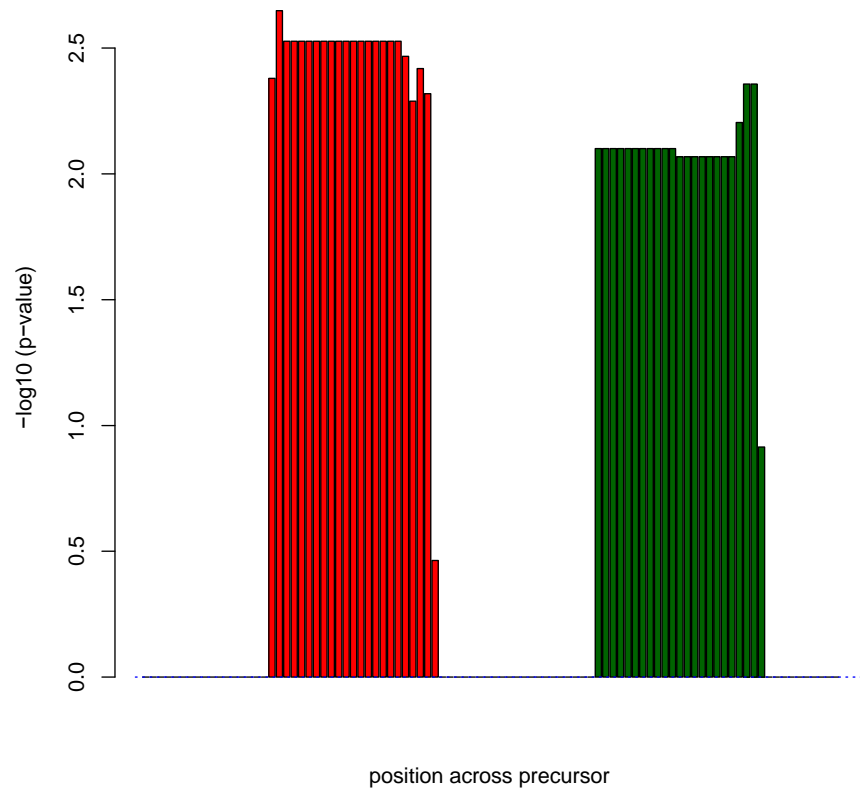
1) Pileup plots



These show the precursor as sequence in the middle, cases sample mappings are highlighted above the precursor and controls below. Known 5' mature miRNAs are colored in blue in the text and known 3' forms red. All known mature forms from miRBase are also highlighted by a blue shaded area.

miFRame presents three general types of results graphics.

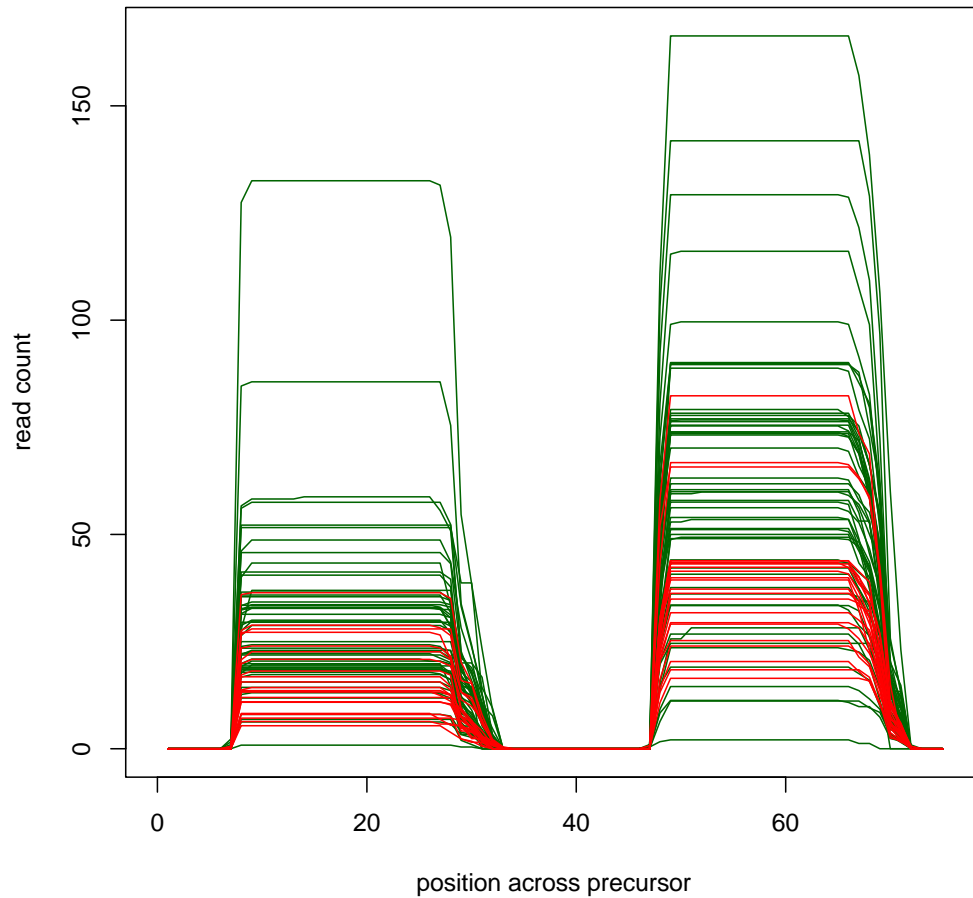
2) Bar plots



The bar plots show for each single position on the precursor whether the respective position is differentially covered in cases and controls. Similar to manhattan plots the negative decade logarithm is presented, red colored bars mean up-regulation and green colored bars down-regulation.

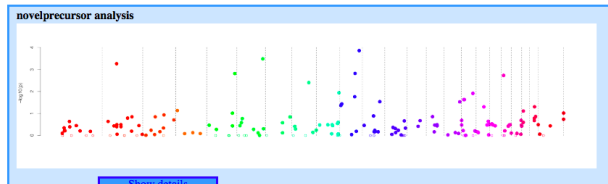
miFRame presents three general types of results graphics.

3) line plots



Here, the coverage of all samples across the precursor is presented. Red lines correspond to controls and green lines to case samples.

In addition to the graphical output, also tabular output is generated. Here, for each miRNA the three plots are presented as thumbnails and different statistical values (e.g. p-values) are presented per miRNA. The data tables can be sorted with respect to the different parameters.



miRNA	length	mean on precursor	mean on precursor AD	mean on precursor AD_control	fold change	p-value	barplot	lineplot	pileup
<input checked="" type="checkbox"/> hsa-mir-103a-1	24	0.75	1.11	0.22	4.99	0.00153			
<input checked="" type="checkbox"/> hsa-mir-107	24	0.7	1.11	0.22	4.96	0.00151			
<input checked="" type="checkbox"/> hsa-mir-3163	23	0.14	0.23	0.05	4.43	0.13			
<input checked="" type="checkbox"/> hsa-mir-181b-2	22	0.2	0.37	0.11	3.36	0.287			

If aggregation analysis has been carried out, for each miRNA and each feature that supports this analysis a pie chart is presented. This pie chart describes in how many other studies the respective miRNA was observed.

Full blue pies mean 100% and white pies mean 0% of studies with the respective finding.

