Tutorial:

miRNACon

You have carried out a case control study for miRNAs in blood and you are not sure on the influence on confounding variables on your signature – **miRNACon** is your tool.

miRNACon estimates the influence oft he most obvious confounding variables, age and gender, on miRNA patterns generated by microarrays and Next Generation Sequencing. To test your signature does not take more than five minutes and five easy steps. Most parameters are already pre-defined in a reasonable manner but can be adapted by users.

Features:

- Support of all miRBase versions starting v16.
- Mapping to the newest version of the miRBase.
- Dynamic calculation of cohorts according to the users requirement
- Easy and well interpretable output
- Multi Threading

This tutorial leady you through the five steps and explains the output. In case of any questions contact us at mirnacon@ccb.uni-saarland.de.

1. Select miRBase version
2. Select the identifier type
3. Chose parameters
4. Select biological source
5. Upload your signature

And now, get your results in few seconds.

Step 1/5:

In the first step you can select between the various miRBase versions using a drop-down menu. Please choose the same version you applied for your experiments as well.

Step 2/5:

Select miRNA identifier ?

⊙ miRBase ID
◯ miRNA sequence

[Proceed]

In the second step you can select whether you want to upload the miRBase IDs or miRNA Sequences.

Step 3/5:

Select parameter ⑦

Requested significance threshold

`0.05`

Requested flooring

`0.001`

Requested average age

`60`

Requested standard deviation of age

`10`

[Proceed]

In the third step, parameter selection happens.

- at which alpha level, miRNAs should be classified as significantly influenced (default: 0.05)
- Very low miRNA p-values may lead to inadequate graphical representation. miRNAs beyond this flooring parameter are set to the flooring parameter (default: 10^{-3})
- Define the average age of your case-control cohorts
- Define the standard deviation of age in your case-control cohorts

Step 4/5:

Select probe type ?

◉ Blood
◯ Serum (TESTING)
◯ Tissue (TESTING)

[Proceed]

In the fourth step you can select the biological source. Currently, just Blood is available, serum and different tissues will follow.

Step 5/5:

1 2 3 4 5

Place your input ⑦

```
hsa-miR-1
hsa-miR-127-5p
```

Calculate

In the last step you have to input your sequences / IDs from your case control study and the calculation can start

Number of patients: 43

Average age: 58.93

Standard deviation of age: 9.21

miRNA Input	miRNA V20 ID	miRNA sequence	potential impact by GENDER	potential impact by AGE
hsa-miR-1	hsa-miR-1	UGGAAUGUAAAGAAGUAUGUAU	-	-
hsa-miR-127-5p	hsa-miR-127-5p	CUGAAGCUCAGAGGGCUCUGAU	-	-
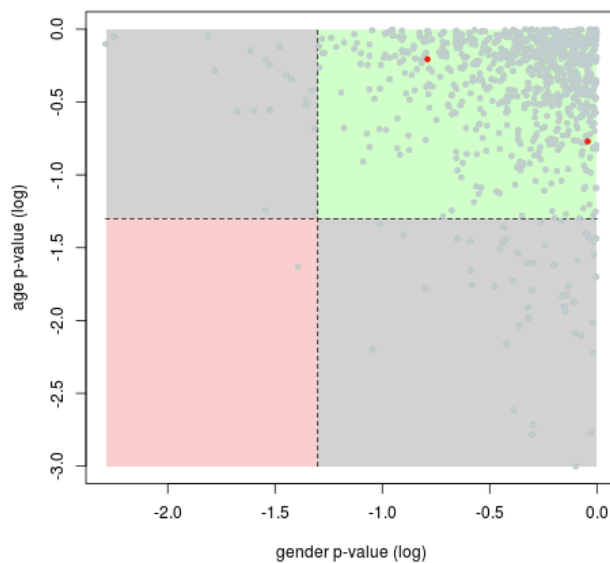
As output you get first a summary: how many patients are selected by miRNAcon, what is the average age and standard deviation of age. The latter both values should be very close to your actual input.

Second, you get a list of you miRNAs, mapped to the most recent version of the miRBase and whether these miRNAs are influenced by age or gender.

Finally, you get a graphical representation. Here, p-values for gender and age are shown, background distribution of all miRNAs as grey dots and your input miRNAs as red dots. The miRNAs in the upper right quadrant are not influenced, in the lower right are influence by age, in the upper left are influenced by gender and in the lower left by age and gender. P-Values are on log-scale.