Manual for



"AmiRNA Designer"



Poznan University of Technology INSTITUTE OF COMPUTING SCIENCE

1. Perspectives

The application works in two modes (perspectives). Each mode corresponds to one of the two design steps of artificial microRNA.

First of prospects called "Data Load" corresponds to the first processing step. In this mode, the application shows the window which provide tools for the management of the data needed to carry out the next tasks.

To select the appropriate perspective, choose in the application main menu option "Perspective" and then the interesting one from the submenu.

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	reMicro td details miKna-preMicro graph			
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			miRna-target	td details mif
				_
		Save Statistic Data		
	\$	Save Statistic Data		
progress		Save Statistic Data		

Figure 1. Window of Data Manager.



The second menu option *"Perspective"* switches the application window to the next processing stage and offers the necessary tools.

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	STa AmiRNA Nucleotide Position A 21 U 12 C 2 G 9	Description	Delete Delete	Load File Kara MiRNA Studio File Perspective Data Load Design FASTa AmiRNA

Figure 2. Application window in design mode.

The AmiRNA Designer allows to import data from different extensions of MS Excel (xls, xlsx, csv), txt and files containing prepared earlier statistics. Imported data should be prepared in accordance with the description of the input data (www.cs.put.poznan.pl/arybarczyk/AmiRNA/InputDescription.pdf).

To start the process of importing data, choose the "Data" option in the view "Data Load". Then, from the menu select the format of the data you are interested in. You can choose the data containing the miRNA, the targets and premiRNA.



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Figure 3. Import miRNA data.

Data Perspective		
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Figure 4. Import target data.

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Figure 5. Import premiRNA data.

AmiRNA Designer allows the user to review and edit the data during the import process itself.

At the beginning of the import process, the user can decide whether he or she is going to edit the data.





Figure 6. Editing the imported data.

All the information connected with the application execution are displayed in the console window. It can be used to check wheteher the input data are correct and the data import process ended without errors.

tive	
A-target Sprocess preMicro Draw Clean Data Loaded miRNA file from: Loaded miRNA file from: Loaded miRNA file from: D:\PP\rma2\doc\ath-miRNA_test.csv	MiRNAlength filter:

Figure 7. Console window.



All imported data can be deleted using the "clean data" button. It applies to "Load Data" perspective.

miRNA Studio	Annalastic Mount Frankish
ile Data Perspective	
Second Se	Separate Process preMicro Draw Clean Data All data cleaned to continue please load agin.
miRna-target td	details miRna-preMicro td details mil Clean recent data



2. Generate thermodynamic details.

The next processing step is dedicated to determine the hybridization temperatures, and to generate statistics needed in the second stage of design process.

Statistics can be generated by running the microRNA:target and preMicro procedures separately.



The message generated by the application when the statistics are ready is as follows:

Data Persp	pective	e																												
🔓 Process mil	RNA-t	arget		5	Process	preMic	ro	1	Draw		Clear	n Data	A	ll data clea	ned to	contin	ue plea	ise load	l agin.Lo	aded m	niRNA 1	ile from		*	MiRNA	length	filter:			
	miR	na-targ	jet td d	letails	miRna	-preMic	ro td d	etails	miRna-	preMic	ro grap	h																		
Gene		0 -0,04	1 -2,05	2 -2,5	3 -2,16	4 -1,58	5 -1,88	6 -2,11	7 -2,1	8 -2,01	9 -1,87	10 -1,9	11 -1,88	12 8 -1,91	13 -1,89	14 -1,92	15 -1,9	16 -1,67	17 -1,59	18 -1,63	19 -1,84	20 -1,91	21 -1,77	22 -1,36	23 -1,18	24 -1,92	25 -2,28	26 -0,84	27 28 0,35 0,	3 29 · 19 -0,
niR156a-"AT2G3	3810	0	-2,07	-2,73	-2,71	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-2,44	-1,81	-1,55	-1,39	-2,38	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		-
niR156g-"AT2G3	3810	0	-2,07	-1,82	-0,8	-0,44	-1,52	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-2,44	-1,81	-1,55	-1,39	-2,38	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
niR156a-"AT1G2	7360	0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR157a-"AT1G2	7360	0	-2,07	-2,73	-2,36	-1,8	-2,08	-2,51	-2,51	-2,23	-2,35	-2	-10	Data loade	ł	-					x	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54	
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niR156g-"AT1G2	27360	0	-2,07	-1,24	0,35	0,72	-0,94	-2,51	-2,23	-2,35	-2	-2	-2		loadii	og file f	rom Di		2) doc)	Coursed		-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
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niR156a-"AT1G5	3160	0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	~	data l	oaded						-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
niR157a-"AT1G5	3160	0	-2,07	-1,82	-0,8	-0,23	-1,43	-2,51	-2,51	-2,23	-2,35	-2	-2									-2,91	-2,71	-1,79	-0,23	-0.8	-1,82	-0,77	0,54	
niR157d-"AT1G5	3160	0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2								_	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
niR156g-"AT1G5	3160	0	-2,07	-1,26	0,31	0,67	-0,97	-2,51	-2,23	-2,35	-2	-2	-2							ОК		-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
niR156h-"AT1G5	3160	0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2-	2,55	0,77	0,10	4,55	6,61	6,00	6,67		-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
niR156a-"AT1G6	9170	0	-0,8	-1,46	-1,44	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR157a-"AT1G6	9170	0	-2,07	-2,88	-2,33	-1,76	-1,9	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54	
niR157d-"AT1G6	9170	0	-0,8	-1,46	-1,44	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2, 11	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR156g-"AT1G6	69170	0	-2,07	-3,19	-2,19	-1,82	-1,54	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,74	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR156h-"AT1G6	9170	0	-0,8	-1,46	-1,44	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR156a-"AT5G5	0570	0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2, 8	-2,33	-2,9	-2,83	-0,77	0,54		
niR157a-"AT5G5	0570	0	-2,07	-1,82	-0,8	-0,23	-1,43	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54	
niR157d-"									_	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	2,33	-2,9	-2,83	-0,77	0,54		
niR156g-"	201	110	oto	tic	tia					-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
niR156h-"	Sa	ve	sta	us	ucs	\$				-2,35	-2	-2	-2	-1,35	-0,44	-0,16														
niR156a-"AT3G1	5270	0	-2,07	-2,73	-2,71	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	C	'alc	nıl	atic	ns	ar	e fi	inis	she	d v	vitl	וא ר	1006	226
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Figure 9. The window with thermodynamic details.

Data Pelspe	Luve						_																	MIRNA	langth	filter			
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	miRna-tar	get td d	etails	miRna	-preMic	ro td d	etails	miRna-	preMic	ro grap	h																		
Gene	0 -0.04	1 -2.05	2 -2.5	3 -2,16	4 -1.58	5 -1.88	6 -2,11	7 -2,1	8 -2.01	9 -1,87	10 -1.9	11 -1.88	12 -1.91	13 -1.89	14 -1.92	15 -1.9	16 -1.67	17 -1.59	18 -1.63	19 -1.84	20 -1.91	21 -1,77	22 -1,36	23 -1.18	24 -1.92	25 -2.28	26 -0.84	27 0.35	28 29
R156a-"AT2	10 0	-2,07	-2,73	-2,71	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-	-2,27	-2,55	-2,27	-2,44	-1,81	-1,55	-1,39	-2,38	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R156g-"AT2G3	10 0	-2,07	-1,82	-0,8	-0,44	-1,52	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-2,44	-1,81	-1,55	-1,39	-2,38	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R156a-"AT1G273	60 0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,4.	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R157a-"AT1G273	60 0	-2,07	-2,73	-2,36	-1,8	-2,08	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	- 35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54	
R157d-"AT1G273	60 0	2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156g-"AT1G273	60 0	-2,47	-1,24	0,35	0,72	-0,94	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,4.	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156h-"AT1G273	60 0	-2,07	1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	- 35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156a-"AT1G531	.60 0	-2,07	-1,1	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	0,16	-0,44	-1,35	-2,91	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R157a-"AT1G531	.60 0	-2,07	-1,82	0,8	-0,23	-1,43	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,5-	-2,27	-2,55	-2,27	-2,91	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54	
R157d-"AT1G53:	.60 0	-2,07	-1,78	-1,15	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-4,55	-2,27	-2,91	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R156g-"AT1G53:	.60 0	-2,07	-1,26	0,31	67	-0,97	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	1,35	-2,91	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R156h-"AT1G531	.60 0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,2,	-2,91	-2,71	-1,79	-0,23	-0,8	-1,82	-0,77	0,54		
R156a-"AT1G691	70 0	-0,8	-1,46	-1,44	-2,35	2.51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-4,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R157a-"AT1G691	70 0	-2,07	-2,88	-2,33	-1,76	-1,9	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54	
R157d-"AT1G69:	.70 0	-0,8	-1,46	-1,44	-2,35	-2,51	.251	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,7-	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156g-"AT1G691	.70 0	-2,07	-3,19	-2,19	-1,82	-1,54	-2,5.	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-4,98	-2,33	-2,9	-2,83	-0,77	0,54		
R156h-"AT1G691	.70 0	-0,8	-1,46	-1,44	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156a-"AT5G505	70 0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R157a-"AT5G505	70 0	mi	RN	a ^₀ åι	n∂l³i	t∳4₹	ařø	e ^{†51}	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	The	-291	imh	éř	and	th	^{2,83} م	rer;	a054	
R157d-"AT5G505	70 0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	2,9	-2,83	-0,77	0,54	18C	
R156g-"AT5G505	70 0	-2,07	-1,26	0,31	0,67	-0,97	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	ten	npe	rati	ure	for	ag	ive	n₅p	air	
R156h-"AT5G505	70 0	-2,07	-1,78	-1,76	-1,4	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-1,35	-0,44	-0,16	-1,35	-2,27	-2,55	-2,27	-2,91	-2,71	-2,88	-2,33	-2,9	-2,83	-0,77	0,54		
R156a-"AT3G152	70 0	-2,07	-2,73	-2,71	-2,35	-2,51	-2,51	-2,23	-2,35	-2	-2	-2	-2,27	-2,55	-2,27	-1,35	-0,16	-0,44	-1,35	-2,91	-1,29	0,06	2,05	0,06	-1,39	-0,77	0,54		
21574_"ATRG15	70 0	.2.07	.273	.271	.2 35	-2.51	.2 51	.2.23	.235	.?	.?	-?	.1 35	-0.44	.016	.1 25	.2 27	.2 55	.2 27	.7 01	-1 70	0.06	2.05	0.06	.1 20	-0 77	0.54		•
												\$		Save S	itatistic	Data													

Figure 10. Description of the the window with thermodynamic details.



The description of a table with thermodynamic details is presented above.

In the text box named "MiRnaLength filter", which is marked in the above Figure, user should type the filter value in the format D, D or DD where d is an integer value (int). This value represents the length of the molecules that will be considered for further processing.

Eg. if the user introduces 19,20, 21-23 ranges, then the molecules of length 19,20,21,22,23 will be taken into account. The default filtering value is empty, which means that all molecules are taken into account (no filtering).

Other statistical parameters not visible in the table such as: maximum value, minimum temperature, next quartiles can be viewed by saving them into a file using the "Save Statistic Data" button.

3. Graphical representation of thermodynamic data.



The application presents the results in the form of "box plot"/", candle stick plot". To generate the graphical representation od the data select "Draw" button.

Figure 11. "Draw" button.



The box plot is generated dynamically and it is possible to freely zoom in and out the interesting parts of the graph. The brief description of the plot is presented below.



Figure 12. The description of the elements of box plot.



Figure 13. Navigate through the box plot.



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4. Application database management

Generation of the statistical data can be time consuming especially for large instances. Therefore, the application contains its internal database to which the user can save or read the thermodynamic data generated previously.

This can be done through "Manage DB" option in "Data" menu. There are "Save" and "Load thermodynamic details" options. The user should choose one of them and next (similarly as in a case of data import) select the type of data he or she is interested in namely miRNA:target or miRNA:premiRNA.



Figure 14. Application database management - menu.

The database initially stores the default set of statistical data. Saving the new set of data will overwrite the previous one.



5. Exporting the thermodynamic data.

The exporting data process is very intuitive. It is the same as in the case of writing information to the database. It is possible to save data in default location or to choose another one.



Figure 15. Data export.

Select "No" in the above dialog window to choose your own location of the file with the exported data.

This file can be then used during the thermodynamic data import process.

6. Import thermodynamic data.

Importing data is done very similarly to the export process. Select the following options: "Data", next "Manage DB" and "Load termodynamic details".

The user will be asked then if he/she would like to import data from database or another sources.



Figure 15. Data import.



Select "No" in the above dialog window to choose your own location of the file you are to import.

7. Designing of the artificial micro RNA [MiRNA*].

The design process takes place in the "Design" perspective.

7.1. Load the file containing data in FASTA format

To load a file in FASTA format use "Load File" button in "FASTA" tab. The content of the file will be shown in console window.

	[NM_202954.3] Arabi	dopsis thaliana gua	nine nucleotide	binding protein	i subunit beta (AGB1)	~
RNA, complet	e cds					
EQUANCE:						- 11
AGUCUCAGGU	ICAGAGAAGCCAUCA	UCAACAUUCAACA	AGAGAGCCGUG	UUUGUGUCUU	GACUGAUUCUUCUC	UC
AGCUUUUUU	AUCUCUCUCUCUUU	UCCCACGUAAUUC	CCCCCAAAUCCA	UUCUUUCUAG	GGUUCGAUCUCCCU	00
UCAAUCAUGA	ACCUUCUUCUCUUCI	UAGACCCCACAAA	GUUUCCCCCUU	JUAUUUGAUCO	GCGACGGAGAAGCO	CUA
GUCUGAUCCO	GGAAUGUCUGUCUC	CGAGCUCAAAGAA	CGCCACGCCGU	CGCUACGGAG	ACCGUUAAUAACCUC	CG
GACCAGCUUA	GACAGAGACGCCUC	CAGCUCCUCGAUA	CCGAUGUGGCG	AGGUAUUCAG	CGGCGCAAGGACGU	AC
CGGGUGAGCU	IUCGGAGCAACGGAU	CUGGUUUGUUGU	CGUACUCUUCA	GGGACACACCO	GAAAGGUUUAUUCA	AU
AGAUUGGAC/	CCGGAGAGGAACCG	GAUUGUCAGUGCA	AUCUCAAGAUG	GAGAUUAAUC	GUGUGGAAUGCUCU	JAA
GAGUCAGAAA	ACUCAUGCUAUUAA	ACUCCCUUGUGCA	UGGGUUAUGAG	AUGUGCUUUC	UCUCCAAAUGGUCA	GU
	AAGAAUGCUCACUG	GUCACAGGGGAUM	UGUUUCGUGC	IGUCAGUAUGI		
	CAGUUCAGGUGAUC			CUACUGGUCU		
GCGGUGAAUI	IUCAGUCUGGACAUA	CUGCUGAUGUACI	JAAGCGUCUCA			uu I
UAUCUGGUUG	AUGCGAUUCCACAG	CACGGUUGUGGGA	ACACUCGUGCUG	SCAAGCCGAGC	AGUGCGUACCUUUC	AU
GUCACGAGG	AGAUGUUAAUACGG	UCAAGUUCUUUC	GGAUGGGUAU	AGAUUUGGGA	CUGGAUCAGACGAU	GG
ACAUGCAGGO	UGUAUGACAUAAGG	ACUGGUCACCAAC	UCCAGGUCUAU	CAGCCACAUG	SUGAUGGUGAGAAC	GG
CCUGUCACCU	CCAUUGCAUUCUCU	GUGUCAGGGAGAC	UUCUUUUCGCU	IGGCUAUGCGA	GCAACAACACUUGC	UA
GUUUGGGAU	ACCCUCUUGGGAGAG	UUUUCUUGCAGG	UUGUAUUGGAU	UUGGGAUUAC	AGCAGGAUUCACAC	4G
AAUAGAAUAA	GCUGUUUGGGGUUG	SUCAGCAGAUGGA	AGUGCCUUGUG	UACAGGAAGUI	JGGGAUUCAAAUCU	AA
GAUAUGGGCC	JUUUGGAGGACACAG	GAGAGUGAUUUG	AAGAAGAUUUA	ACGAAAGUAG	GAGUCACGUCUCCAC	SU
GUUGGUUAAI	JAUAUUCUGUAGUCO	GGGAAGUAAGGUU	CGGUUUGUGGA	AGGUGUUUGG	UUUGAAAUAGUGG/	AG
GGUUAGAAG/	AUUAAACUUCCCUU	UUUGUAGUGUGCI	UUUGAUUUAUU	UAUUUCUUCA	UUGGGAACUAAACU	cc
LICAACACGCU	ACUCAAUGUGAAUU	CUGUAAUCAAUUG	GUGUACCCACCA	GUCUUUACUU	UACUAUCAUCUCUU	C I

Figure 16. FASTA file content in console window.



7.2. Validation of the content of the FASTA file

In the "FASTA" tab, the tool for validation of the FASTA file is located. In the left part of the window, the four default rules are displayed. Each rule is accompanied by a description, containing guidance on its merits. The user decides whether the a given rule is taken into account. Depending on his/her decision the rule should be removed from the list (not taken into account) or left (will be taken into account). To remove the rule from the list use "Delete" button located by each of them.

Additionally, if the default rule set is insufficient for the user, he/she can add his/her own set. Expand the panel located above the table of rules for this purpose.



Figure 17. Validation process.

After setting the parameters and pressing "Add" the rule becomes active.



	<u>P</u> erspective				
a Ami	RNA				🧭 clean Load File
			Validate		
	Position 21 12 2 9	Description		Targets FA5_14: AGGAGCCAUCAUCAACAUUCA FA5_20: CAUCAUCAACAUUCAACAAUGA FA5_82: UUAUCUCUCUCUUUUUUCCCA FA5_88: CUUCUUCUUUUUUCCCAACGUA FA5_99: UUCCCCCCCUCUUUUUCCCAAGUAA FA5_99: UUCCCCUCUUUUUCCAAUCAUGA FA5_140: AUCUCCCUUCUUUUUCAAUCAUGA FA5_186: AAGUUUCCCCCUUUUAUUUUCA FA5_186: AAGUUUCCCCCUUUUAUUUUGA FA5_376: CGUUAAUAACUCCGUGGACCA FA5_376: CGAUGCGGUGAGCUUCGGAGGAGUA FA5_376: CUCGGUGAGCUUCGGAGCAA FA5_376: UGUUGUGUACUUCUUCAGGACA FA5_408: UGUUGUCGUAUCUUCAGGGA GA4	gj13651623]refj1WI, 202954.3] Arabidopsis thaliana guanine nucleotide-binding protein subunit beta (AGB1) mRNA, complete cds SEQUANCE: UAGUULCAGGUUCAGAGAAGCCAUCAUCAACAUUCAACAAGAGAGAG
			•	FAS_439: AGGUUUAUUCAUUAGAUUGGA FAS_474:	

Figure 18. Validation panel.

Validation process starts through selecting "Validate" button. The panel containing the validation results expands. If the user is not satisfied with the results, he/she can clear the data using the "clean" button. The user must then reload the FASTA file and run the validation process with new parameters.

7.3. Design procedure

To begin the next stage of the design of artificial microRNA, select the "AmiRna" tab in "Design" perspective.

It is important to have undergone the steps described in the previous chapters of this manual.

On the left side of the tab there is a panel where the user can change the default parameters of the algorithm.



The "*Process 2nd stage*" button starts the algorithm. This procedure is time consuming and it is possible, that the tab will be temporarily blocked and the "*AmiRNA Preprocessing*" message will be shown.

The results of the processing can be saved using the "Save Result Data" button, or delete using "clean" button.

If there will not be any results generated for the selected parameters of the algorithm, then the table will be empty.

STa AmiRNA					
Process 2nd Stage	clean				
Id AmiRNA	AmiRNA	id AmiRNA*	AmiRNA*	Parameters	
amiR_FAS_56_6G_20G	UUGAGGGAAGAAUCAGUCAGG	antyamiR_FAS_56_6G_20G_16L	CCUGACUGAUUCUUCUCUCAA	Size of the package [mg.	10
amiR_FAS_56_20G	UUGAL 1GAAGAAUCAGUCAGG	antyamiR_FAS_56_20G_16U	CCUGACUGAUUCUUCUCUCAA	Minimal space [int]:	3
amiR_FAS_410_5U_18G	UGUCUCUGAAL YGUACGGCAA	antyamiR_FAS_410_5U_18G_15	UUGCCGUACUCUUCGGAGACA	3rd quartile increase [%]:	0
amiR_FAS_410_5U	UGUCUCUGAAGAGUAGU CAA	antyamiR_FAS_410_5U_15G	UUGUCGUACUCUUCGGAGACA		
amiR_FAS_410_18G	UGUCCCUGAAGAGUACGGCAA	antyamiR_FAS_410_18G_15G	UUGCCGUACUCUUCGGGGACA		
amiR_FAS_483_5U_19G	UCUCUCAUCUUGAGAUGCGCU	anu, miR_FAS_483_5U_19G_16	AC FASTa AmiRNA		
amiR_FAS_483_5U_19G	UCUCUCAUCUUGAGAUGCGCU	antyamiR_FA_ 483_5U_19G_16	AC Process	clean	
amiR_FAS_483_5U_19G	UCUCUCAUCUUGAGAUGCGCU	antyamiR_FAS_483_50_19G_1G	GC 2nd Stage		
amiR_FAS_483_5U_19G	UCUCUCAUCUUGAGAUGCGCU	antyamiR_FAS_483_5U_19G_21	Id AmiRNA	AmiRNA	id AmiRNA*
amiR_FAS_483_5U_20U	UCUCUCAUCUUGAGAUGCAUU	antyamiR_FAS_483_5U_20U_16	Arg		
amiR_FAS_483_5U	UCUCUCAUCUUGAGAUGCACU	antyamiR_FAS_483_5U_16G_21	AC		
amiR_FAS_483_5U	UCUCUCAUCUUGAGAUGCACU	antyamiR_FAS_483_5U_16G	AC		AmiRNA proessing
amiR_FAS_483_5U	UCUCUCAUCUUGAGAUGCACU	antyamiR_FAS_483_5U_21G	AC		
amiR_FAS_864_6U_15G	UGAUCUACUGAUUGGGACGCU	antyamiR_FAS_864_6U_15G_10	GC		
amiR_FAS_864_6U	UGAUCUACUGAUUGAGACGCU	antyamiR_FAS_864_6U_1G	Galestantener	-	
		Save Result Data			

Figure 19. **Design process.**

