Integrate miRNA and Gene Expression data

miRNAs regulate gene expression at the post-transcriptional level by base-pairing with the three prime untranslated region (3' UTR) of the target gene, causing cleavage/degradation of the cognate mRNA or preventing translation initiation. Integration of miRNA expression with gene expression data to study the overall network of gene regulation is vital to understanding miRNA function in a given sample. Partek Genomics Suite provides a platform that can analyze miRNA and gene expression data independently, yet allows data to be integrated for downstream analysis.This integrative analysis can be accomplished at several different levels. If you only have miRNA data, then Partek Genomics Suite can search the predicted gene targets in a miRNA-mRNA database like TargetScan to provide a list of genes that might be regulated by the differentially expressed miRNAs. Alternatively, if you have only gene expression data, Partek Genomics Suite can use the same database to identify the microRNAs that putatively regulate those differentially expressed genes in a statistically significant manner. If you have gene expression data and miRNA data from comparable tissue/species, Partek Genomics Suite can combine the results of these separate experiments into one spreadsheet. Lastly, if the miRNA and mRNA from the same source was analyzed (as in this tutorial), then you may statistically correlate the results of miRNA and gene expression assays.

Finding putative genes regulated by miRNAs

This application is useful in the case where you have miRNA expression data, but not gene expression data. Using a database like TargetScan, microCosm, or a custom database, you can identify the list of genes that are predicted to be regulated by these differentially expressed miRNAs and then perform *Biological Interpretation* tasks on the list of genes.

- Select **Combine miRNAs with their mRNA targets** from the *miRNA Integration* section of the *miRNA Expression* workflow
- Select the **Get All Targets** tab
- Select **TargetScan7.1** for *Database Name*
- Select **brain vs. heart human** for *Spreadsheet Name*
- Set *Column with microRNA labels* to **2. Probeset ID**
- Name the *Result file* **PutativeGenes**
- Select **OK** (Figure 1)

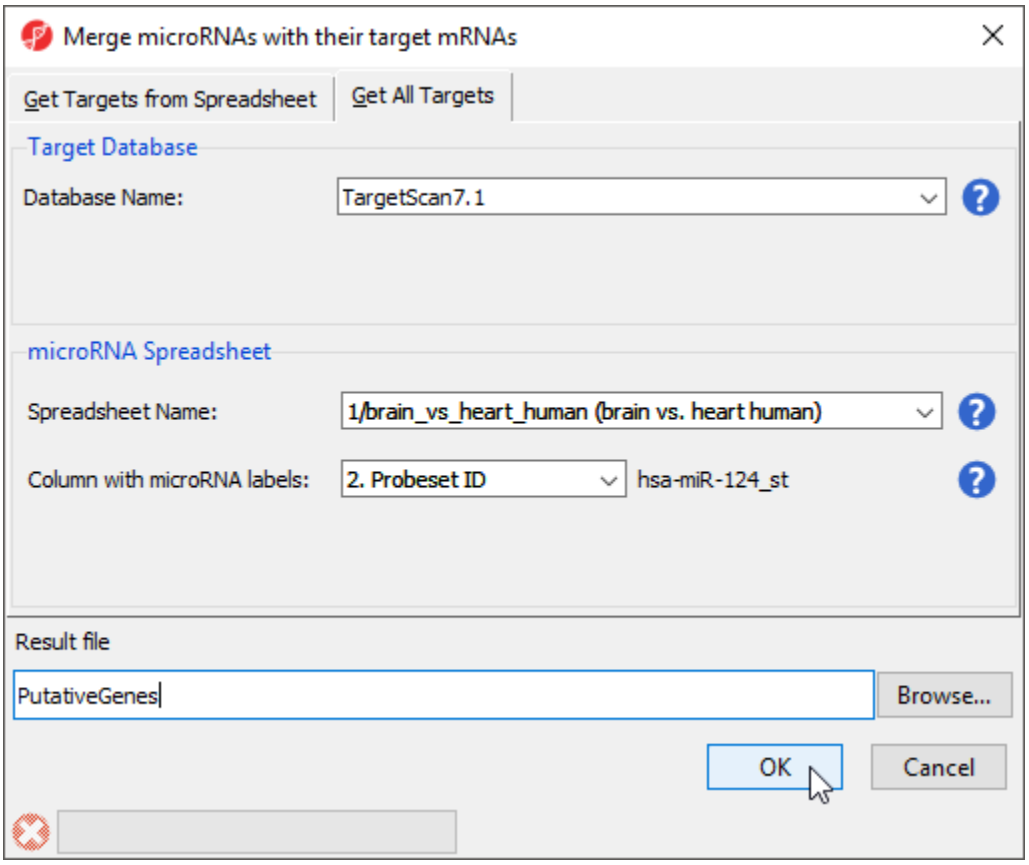


Figure 14. Identifying all predicted gene targets of differentially expressed miRNAs

This will create a new spreadsheet *PutativeGenes* that contains a miRNA and a putative gene target in each row. Because each miRNA can regulate multiple genes, the list will be much longer than the input miRNA list. Each row contains a gene so this spreadsheet can be analyzed using GO Enrichment and Pathway Enrichment tasks from the *Biological Interpretation* section of the workflow.

Another useful way to analyze this list is to determine which genes could be targeted by multiple miRNAs in the list. To do this:

- Right-click on the column *13. Gene Symbol* header
- Select **Create List With Occurrence Counts** from the pop-up menu (Figure 2)

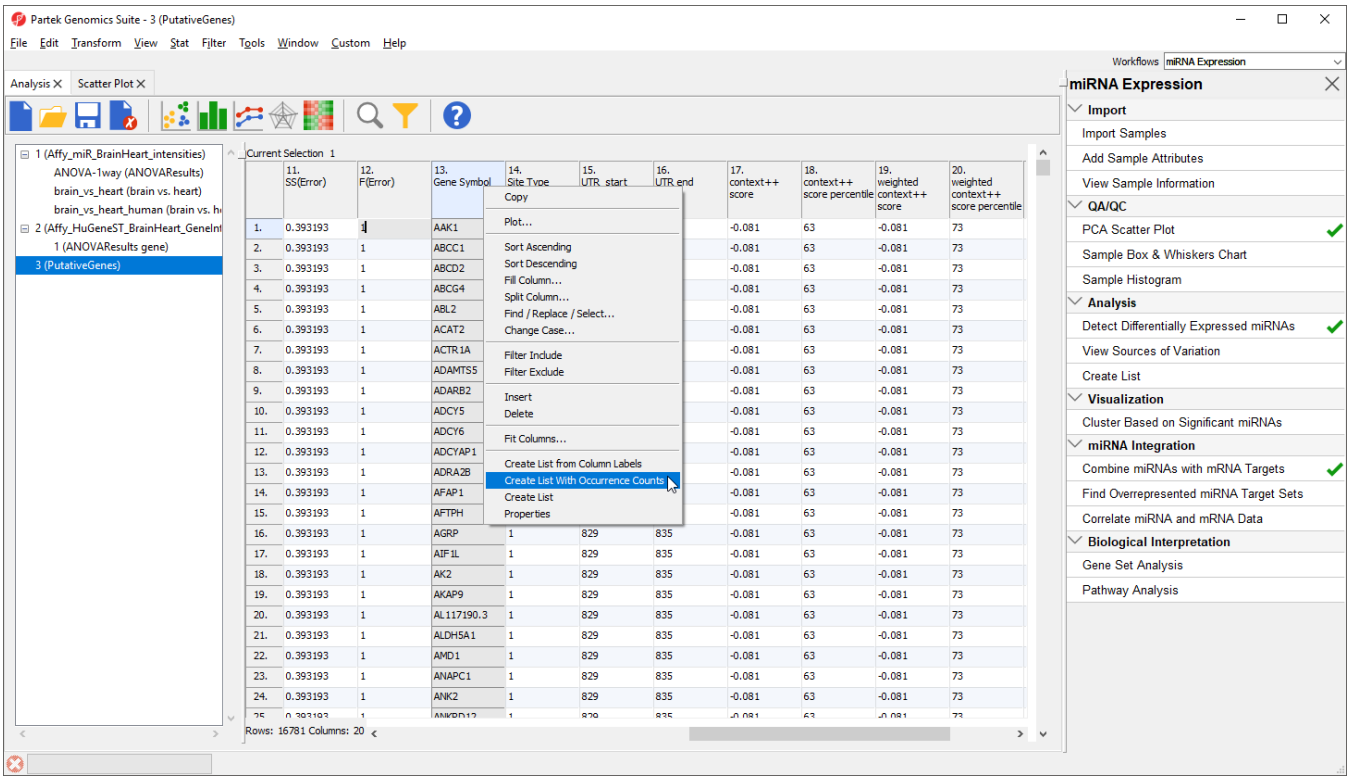


Figure 15. Creating an occurrence counts list from the list of putative miRNA target genes

The new spreadsheet is a temporary spreadsheet listing each gene in alphabetical order and giving the occurance count of each. Sorting by descending order will list the gene with the most occurances first (Figure 3).

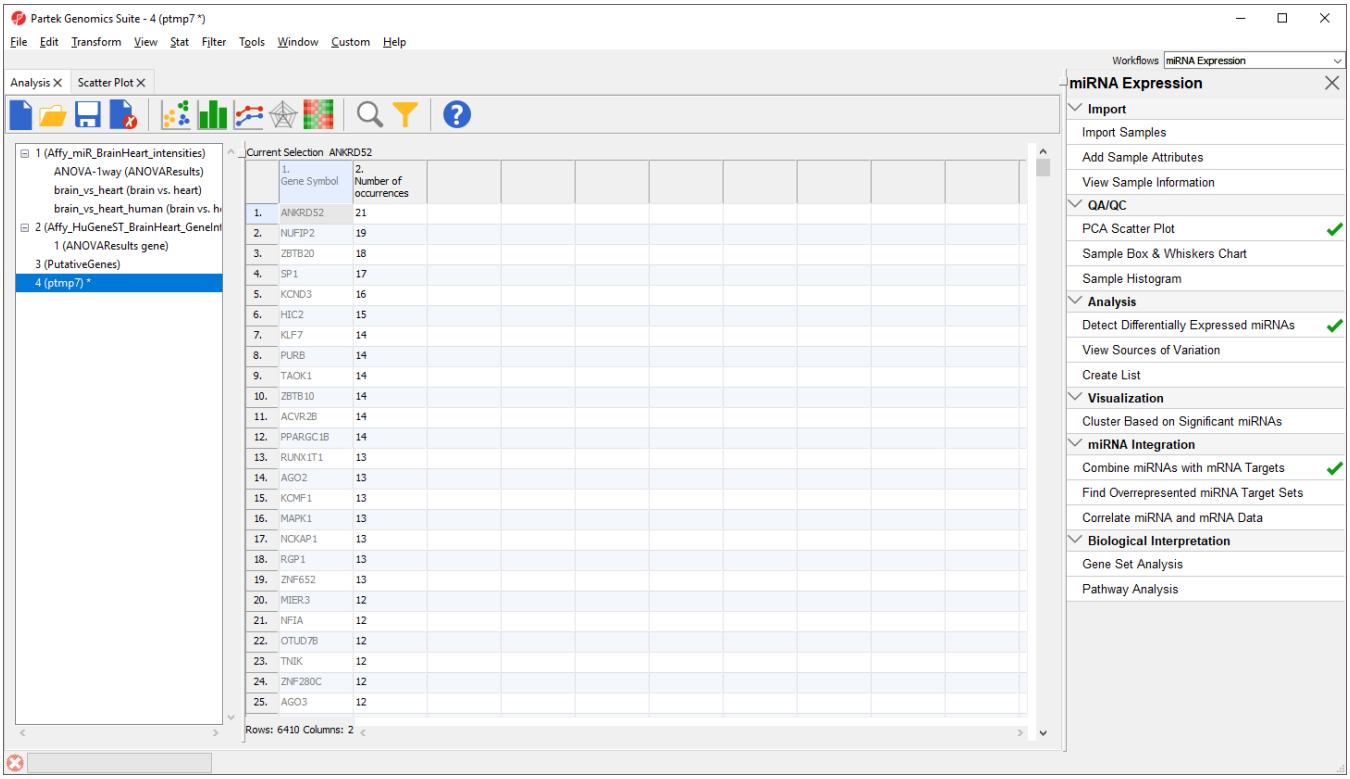


Figure 16. Occurrence list of putative miRNA target genes

We will not be using this temporary spreadsheet moving forward. You can close the spreadsheet by selecting 

Finding overrepresented miRNA targets sets from gene expression data

This application is useful when you only have gene expression results or a gene list of interest and are interested in identifying which miRNAs might regulated the genes. Using a databse like TargetScan, you can create a list of miRNAs that are statistically predicted to regulated those genes. miRNAs of particular interest could then be explored using a lower-throughput technique like RT-qPCR.

Using the gene list as input, a Fisher's Exact right-tailed p-value is calculated to show the overrepresentation of genes of interest for each miRNA in the database. The smaller the p-value, the more overrepresented the miRNAs are for the dataset. Target associations are taken from a database, TargetScan in this example. If the input list is a filtered list of genes from an ANOVA calculation, the parent spreadsheet is used to identify the background list of genes from the array. Genes in the array but not in the significant gene list will be treated as background in the calculations.

To begin, we need to create a list of significant genes using the *ANOVAResults gene* spreadsheet.

- Select the *ANOVAResults gene* spreadsheet in the spreadsheet tree
- Select **Create List** from the workflow
- Select **Brain vs. Heart**
- Set the *Save list as* to **brain vs. heart genes**
- Leave other fields at their default values (Figure 4)
- Select **Create**

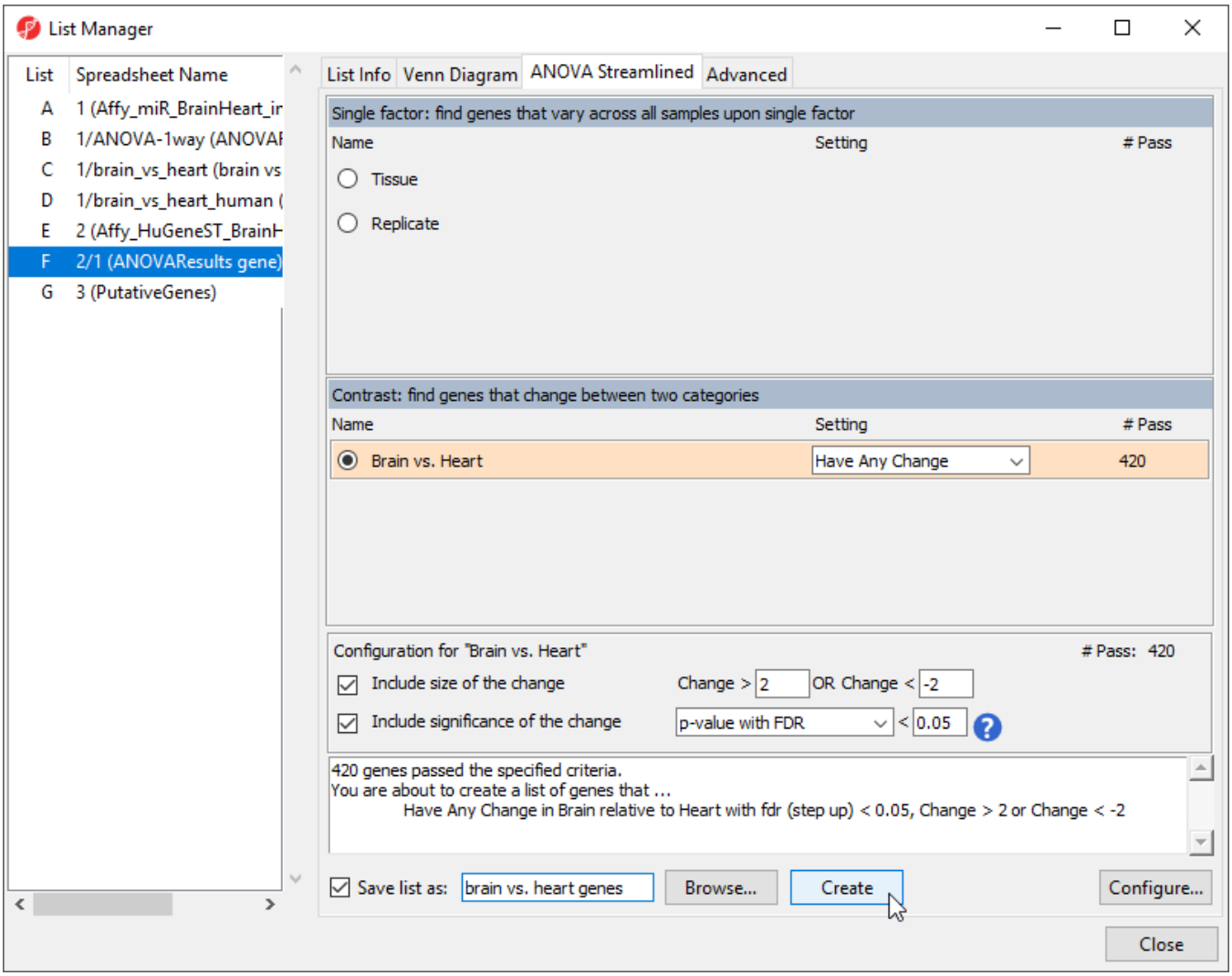


Figure 17. Creating a list of significantly differentially expressed genes

- Select **Close** to exit the *List Manager* dialog

We will now use this list to identify overrepresented miRNA target sets.

- Select **Find overrepresented miRNA target sets** from the *miRNA Integration* section of the workflow
- Select **TargetScan 7.1** from the *Target Databse* drop-down menu
- Select **brain vs. heart genes** from the *mRNA Spreadsheet* drop-down menu
- Select **4. Gene Symbol** from the *Column with gene symbols* drop-down menu (Figure 5)
- Select **OK**

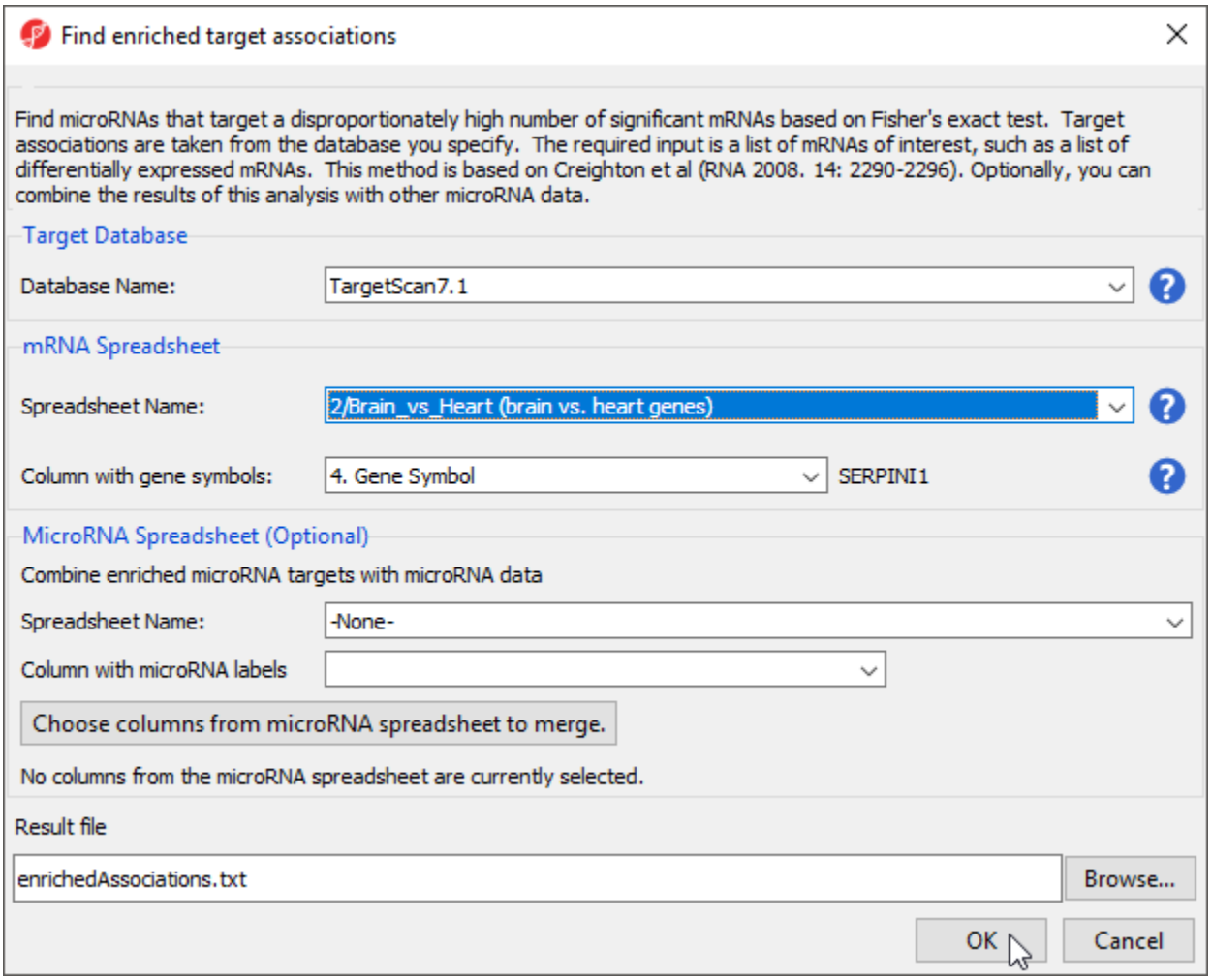


Figure 18. Finding enriched miRNA target sets

A new spreadsheet, *enrichedAssociations,* will be created with miRNAs from the database on rows (Figure 6). Column 1 contains the miRNA name and column 2 shows its p-value. The smaller the p-value, the more significant it is. Column 3 contains the number of genes from the (input) significant gene list that are targeted by this microRNA and Column 7 shows the number of significant genes from the input list that are not targeted by this microRNA. Columns 4 and 5 contain the number of significantly up- and down-regulated genes from the input significant gene list targeted by the miRNA. Column 6 shows the number of background genes (genes on the array but not in the input significant gene list) that are targeted by the miRNA and Column 8 shows the number of background genes on the array that are not targeted by the miRNA. The numbers in columns 3, 6, 7 and 8 will be used to calculate the Fisher's Exact (right-tailed) p-value, a measure of the overrepresentation of the predicted miRNAs within a gene set.

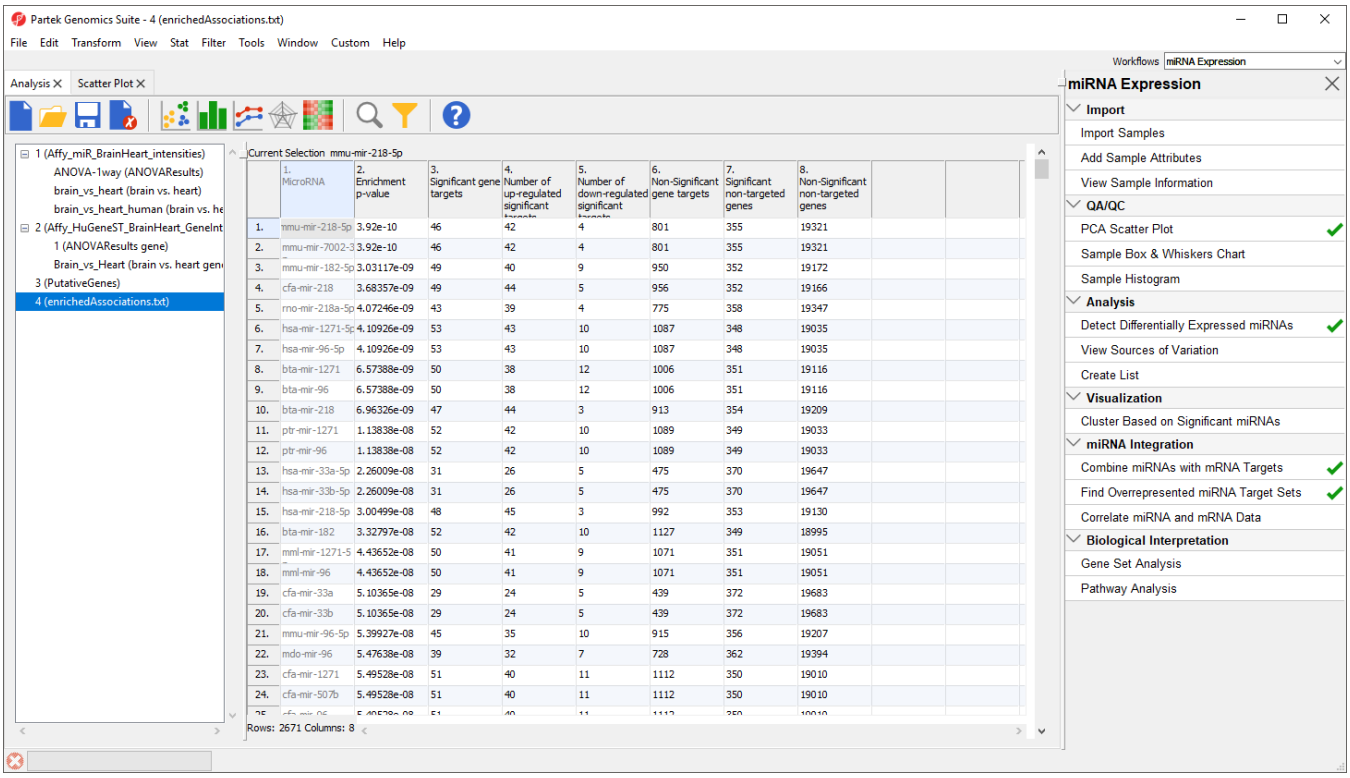


Figure 19. Output of the Find Overrepresented miRNA Target Sets tool

As the enrichment p-values have not been corrected for running multiple statistical tests, we can the multiple test corrrection feature of Partek Genomics Suite to adjust the p-values.

- Select the **enrichedAssociations** spreadsheet
- Select **Stat** from the main menu toolbar
- Select **Multiple Test Correction**
- Select all the multiple test correction options
- Transfer *Enrichment p-value* to the *Selected Column(s)* panel from the *Candidate Column(s)* panel (Figure 7)

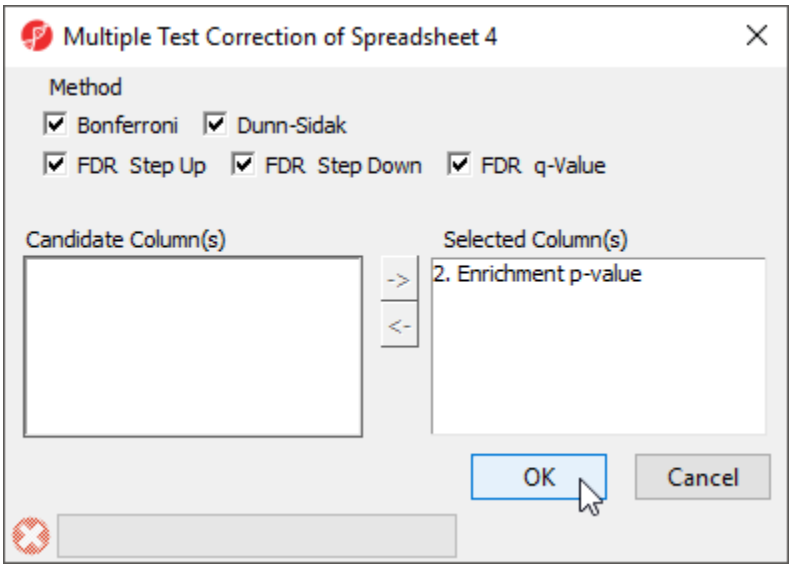


Figure 20. Configuring the Multiple Test Correction dialog

Columns for each of the test correction methods will be added to the *enrichedAssociations* spreadsheet and can be used to filter the list of miRNAs.

Combine miRNAs with mRNA target genes

This option is useful if you have miRNA and gene expression experiments you want to compare. The samples should be comparable, but do not have to originate from the same specimens.

- Select **Combine miRNAs with their mRNA targets** from the *miRNA Integration* section of the workflow
- Select the **Get Targets from Spreadsheet** tab
- Select **TargetScan 7.1** from the *Target Database* drop-down menu
- Select **brain vs. heart human** from the *microRNA Spreadsheet* drop-down menu
- Select **2. Probeset ID** for *Column with microRNA labels*
- Select **ANOVAResults gene** from the *mRNA Spreadsheet* drop-down menu
- Select **4. Gene Symbol** for *Column with gene symbols* (Figure 8)
- Select **OK**

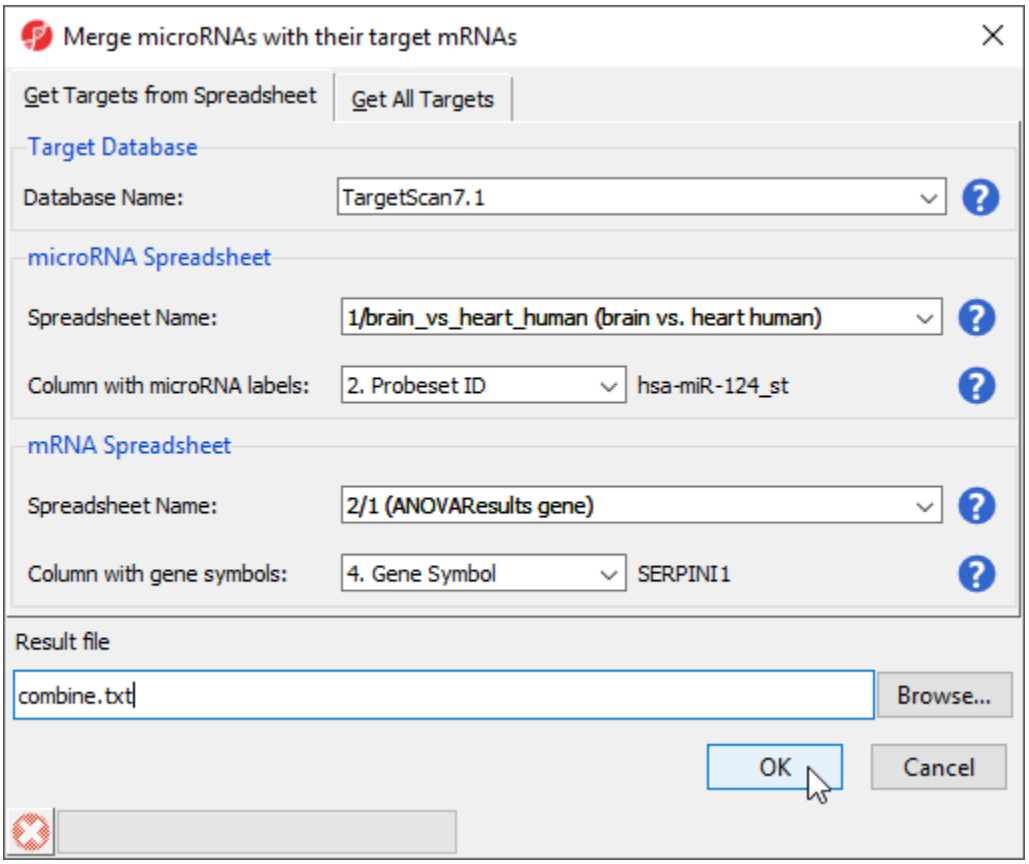


Figure 21. Combining miRNAs with their mRNA targets

In the new spreadsheet, each row represents a specific miRNA associated with one of its target genes; a single miRNA can have multiple targets. For example, *hsa-miR-133b_st* has 659 rows, one for each target (Figure 9).

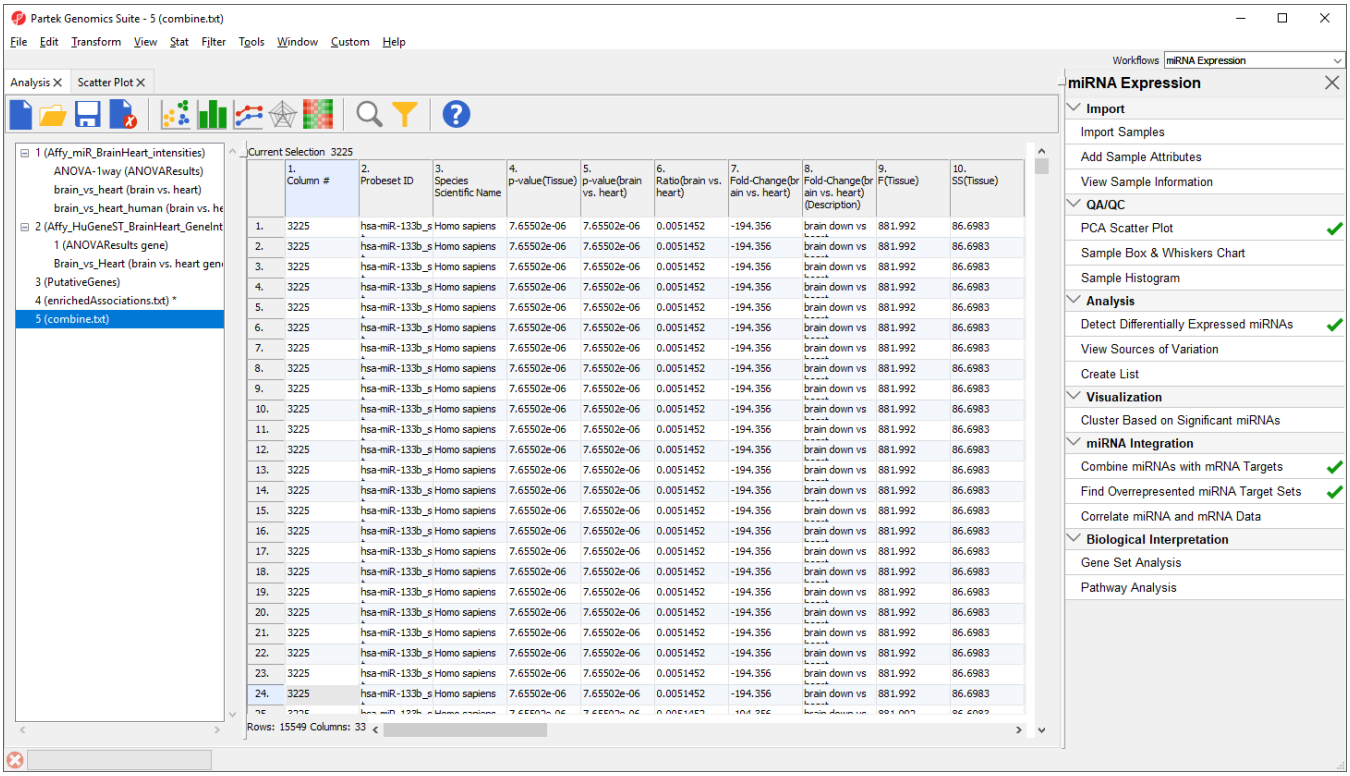


Figure 22. Viewing the combined spreadsheet with miRNAs and mRNA targets

Columns 1-12 are taken from the miRNA expression source spreadsheet while columns 13-26 are taken from the gene expression source spreadsheet.

Correlating miRNA and gene expression data

This application is useful when you have miRNA and mRNA expression data form the same samples and want to correlate the findings to determine whether up- or down-regulated miRNAs result in gene expression changes in their cognate genes. Pearson and Spearman correlation coefficients and their p-values are calculated.

- Select **Correlate miRNA and mRNA data** from the *miRNA Integration* section of the workflow
- Select **TargetScan7.1** from the *Target Database* drop-down menu
- Select **Affy_miR_BrainHeart_intensities** for the *microRNA spreadsheet* using the drop-down menu
- Select **Affy_HuGeneST_BrainHeart_GeneIntensities** as the *mRNA spreadsheet* using the drop-down menu (Figure 10)
- Select **OK**

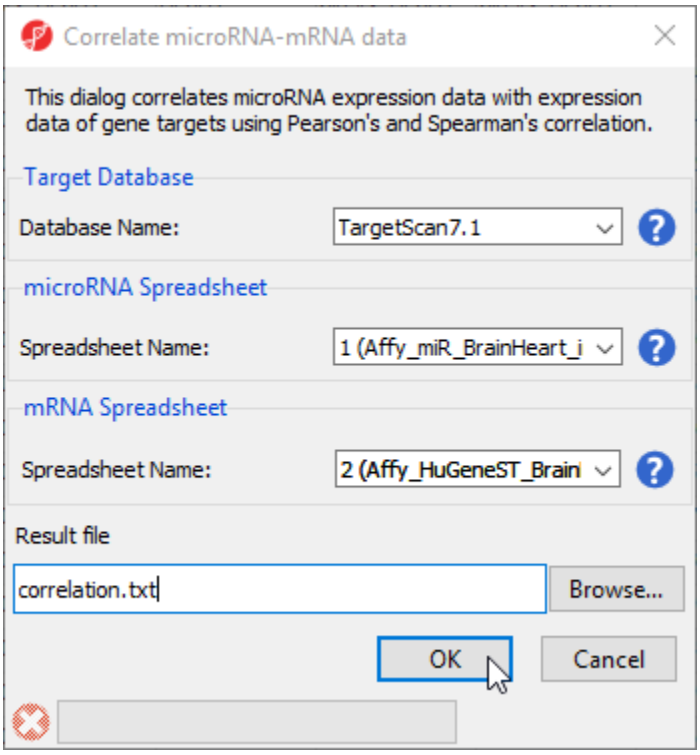


Figure 23. Configuring the Correlate miRNA-mRNA dialog

Next, select the SmapleID column from each spreadsheet. These must match.

- Select **6. SampleID** for *Affy_miR_BrainHeart_intensities*
- Select **6. SampleID** for *Affy_HuGeneST_BrainHeart_GeneIntensities*
- Select **OK** (Figure 11)

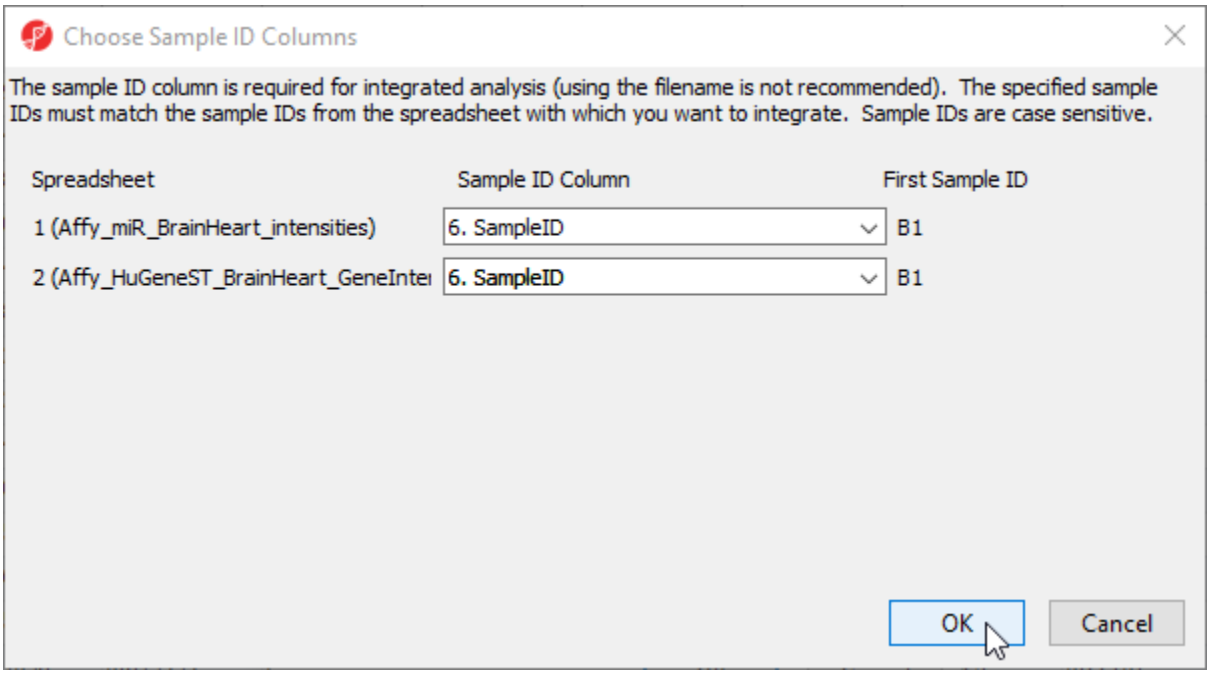
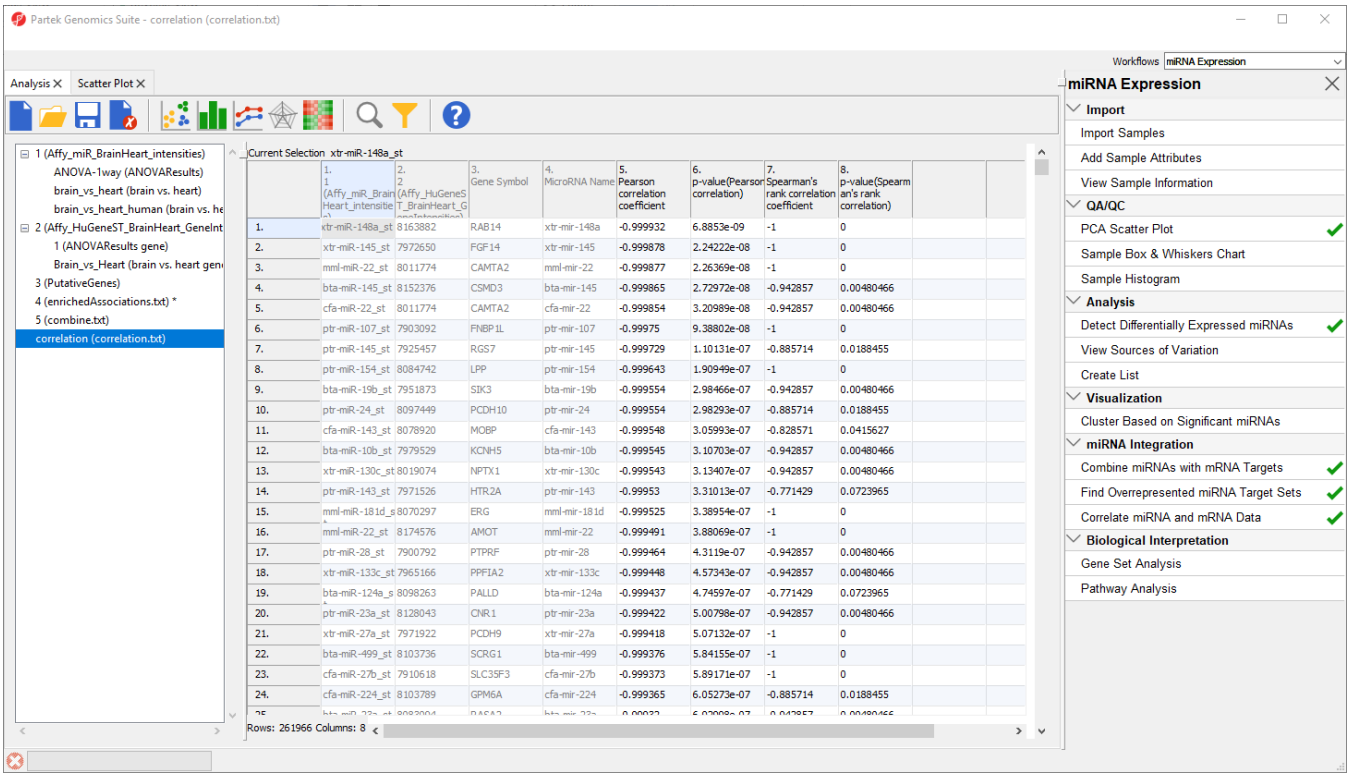


Figure 24. Choosing matching Sample ID columns

The new spreadsheet, *correlation.txt* (Figure 12). Each row contains one miRNA correlated with one of its target gnees. The first column contains the miRNA probeset ID from the miRNA intensities spreadsheet. The second column contains the mRNA probeset ID from the gene expression intensities spreadsheet. The third column lists the gene symbol and the fourth the miRNA name. The fifth and sixth columns are the Pearson correlation coefficient and its p-value for the gene-miRNA pair. The seventh and eigth columns are the Spearman's rank correlation coefficient and its p-value for the gene-miRNA pair. Negative correlation indicates that a high level of the miRNA is correlated with a low expression level in its target gene. Positive correlation indicates that a high level of the miRNA is associated with a high level of its target gene.



	1. 1 (Affy_miR_BrainHeart_intensitie	2. 2 (Affy_HuGeneST_BrainHeart_G	3. Gene Symbol	4. MicroRNA Name	5. Pearson correlation coefficient	6. p-value(Pearson correlation)	7. Spearman's rank correlation coefficient	8. p-value(Spearman's rank correlation)
1.	xtr-miR-148a_st	8163882	RAB14	xtr-mir-148a	-0.999932	6.8853e-09	-1	0
2.	xtr-miR-145_st	7972650	FGF14	xtr-mir-145	-0.999878	2.24222e-08	-1	0
3.	mml-miR-22_st	8011774	CAMTA2	mml-mir-22	-0.999877	2.26369e-08	-1	0
4.	bta-miR-145_st	8152376	CSMD3	bta-mir-145	-0.999865	2.72972e-08	-0.942857	0.00480466
5.	cfa-miR-22_st	8011774	CAMTA2	cfa-mir-22	-0.999854	3.20989e-08	-0.942857	0.00480466
6.	ptr-miR-107_st	7903092	FNBP1L	ptr-mir-107	-0.99975	9.38802e-08	-1	0
7.	ptr-miR-145_st	7925457	RGS7	ptr-mir-145	-0.999729	1.10131e-07	-0.885714	0.0188455
8.	ptr-miR-154_st	8084742	LPP	ptr-mir-154	-0.999643	1.90949e-07	-1	0
9.	bta-miR-19b_st	7951873	SIK3	bta-mir-19b	-0.999554	2.98466e-07	-0.942857	0.00480466
10.	ptr-miR-24_st	8097449	PCDH10	ptr-mir-24	-0.999554	2.98293e-07	-0.885714	0.0188455
11.	cfa-miR-143_st	8078920	MOBP	cfa-mir-143	-0.999548	3.05993e-07	-0.828571	0.0415627
12.	bta-miR-10b_st	7979529	KCNH5	bta-mir-10b	-0.999545	3.10703e-07	-0.942857	0.00480466
13.	xtr-miR-130c_st	8019074	NPTX1	xtr-mir-130c	-0.999543	3.13407e-07	-0.942857	0.00480466
14.	ptr-miR-143_st	7971526	HTR2A	ptr-mir-143	-0.99953	3.31013e-07	-0.771429	0.0723965
15.	mml-miR-181d_s	8070297	ERG	mml-mir-181d	-0.999525	3.38954e-07	-1	0
16.	mml-miR-22_st	8174576	AMOT	mml-mir-22	-0.999491	3.88069e-07	-1	0
17.	ptr-miR-28_st	7900792	PTPRF	ptr-mir-28	-0.999464	4.3119e-07	-0.942857	0.00480466
18.	xtr-miR-133c_st	7965166	PPFIA2	xtr-mir-133c	-0.999448	4.57343e-07	-0.942857	0.00480466
19.	bta-miR-124a_s	8098263	PALLD	bta-mir-124a	-0.999437	4.74597e-07	-0.771429	0.0723965
20.	ptr-miR-23a_st	8128043	CNR1	ptr-mir-23a	-0.999422	5.00798e-07	-0.942857	0.00480466
21.	xtr-miR-27a_st	7971922	PCDH9	xtr-mir-27a	-0.999418	5.07132e-07	-1	0
22.	bta-miR-499_st	8103736	SCRG1	bta-mir-499	-0.999376	5.84155e-07	-1	0
23.	cfa-miR-27b_st	7910618	SLC35F3	cfa-mir-27b	-0.999373	5.89171e-07	-1	0
24.	cfa-miR-224_st	8103789	GPM6A	cfa-mir-224	-0.999365	6.05273e-07	-0.885714	0.0188455

Figure 25. Viewing the correlation spreadsheet

We can visualize the correlation between any miRNA and target gene.

- Right-click a row header
- Select **Scatter Plot (Orig. Data)** from the pop-up menu

The correlation plot shows miRNA intensitiy on the x-axis and gene expression on the y-axis (Figure 13). Here, we see a negative correlation between expression of xtr-miR-148a_st and its target gene, RAB14, in brain and heart tissues. Drawing the scatter plot will create a temporary file with miRNA and gene expression probe intensities for all samples that is used to draw the plot.

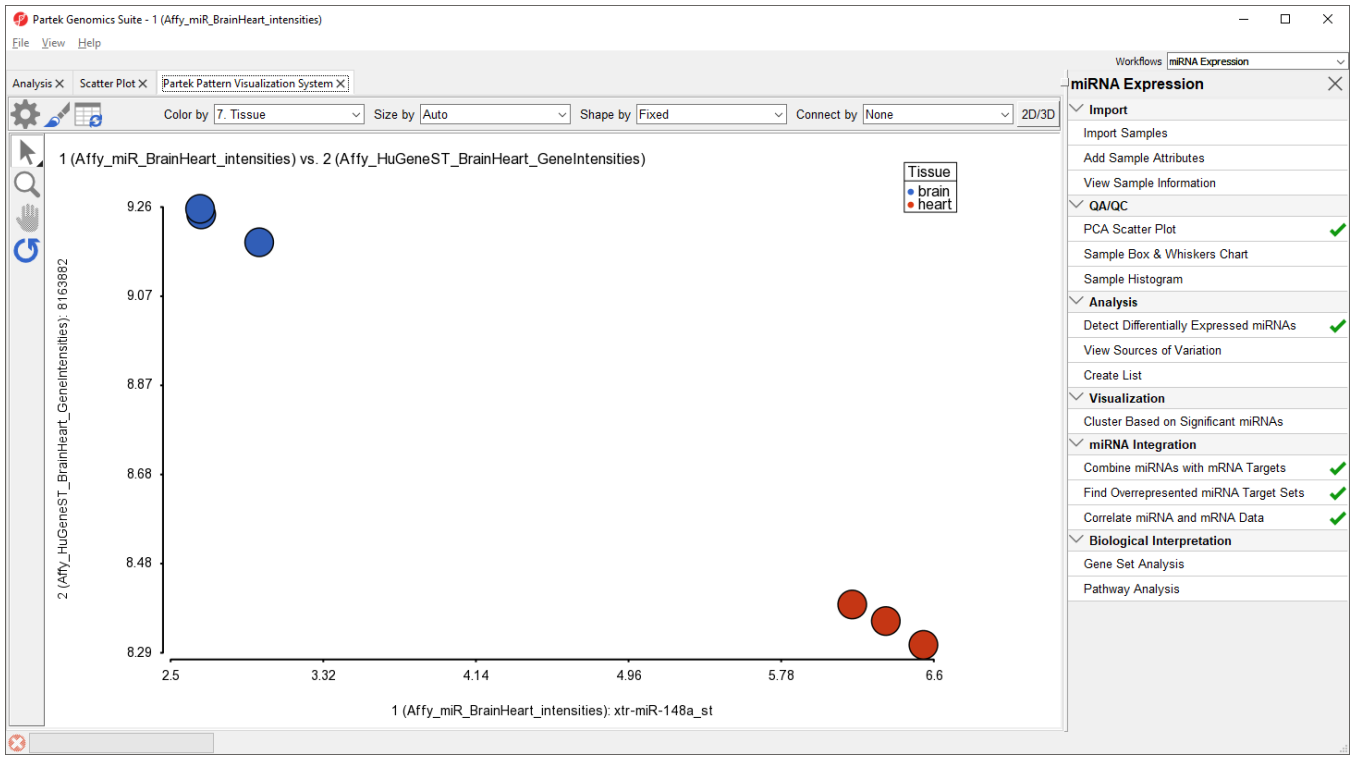


Figure 26. Viewing the scatter plot showing correlated miRNA and target gene expression

Please note that the correlation function is only useful for identifying miRNAs that affect mRNA stability, not translation.

Additional Assistance

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