Analyze differentially expressed miRNAs

- Exploratory data analysis
- Detecting differentially expressed miRNAs
- Creating a list of miRNAs of interest

Typically, you would begin a miRNA expression analysis with the same steps outlined in the Importing Affymetrix CEL files section of the *Gene Expression* t utorial. Here, the data has already been imported and attributes added.

To being our analysis, we will open the *miRNA Expression* workflow.

- Select the **miRNA Expression** workflow from the *Workflows* drop-down menu

The *miRNA Expression* workflow provides a series of steps for analyzing miRNA expression data and integrating it with gene expression data (Figure 1).

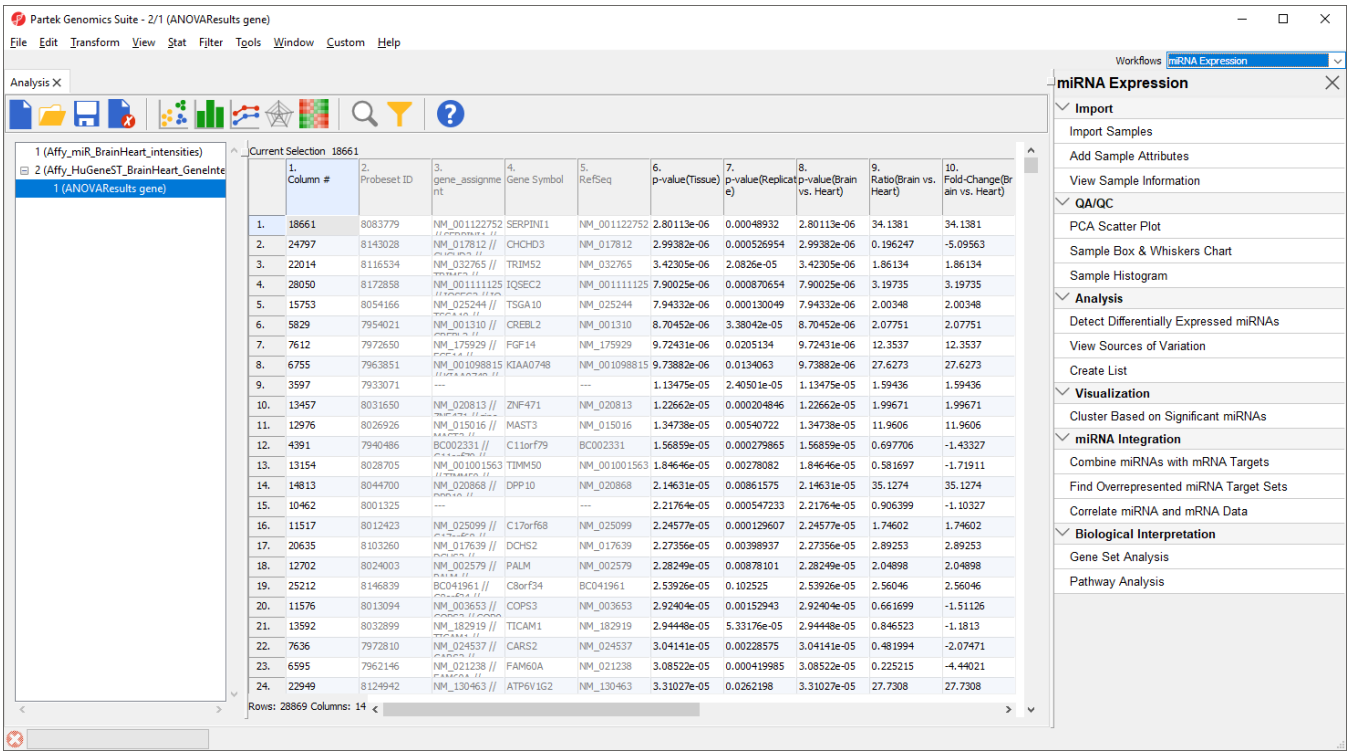


Figure 15. The miRNA Expression workflow

Exploratory data analysis

Principal Components Analysis (PCA) is an excellent method to visualize similarities and differences between the samples in a data set. PCA can be invoked through a workflow, by selecting  from the main command bar, or by selecting Scatter Plot from the View section of the main toolbar. We will use a workflow.

- Select the **Affy_miR_BrainHeart_intensities** spreadsheet

This is the probe intensities spreadsheet for the miRNA expression data (Figure 2). Each row is a sample; columns 7 to 9 give attribute information about each sample including tissue, replicate number, and scan date, while columns 10 on give prove intensities values.

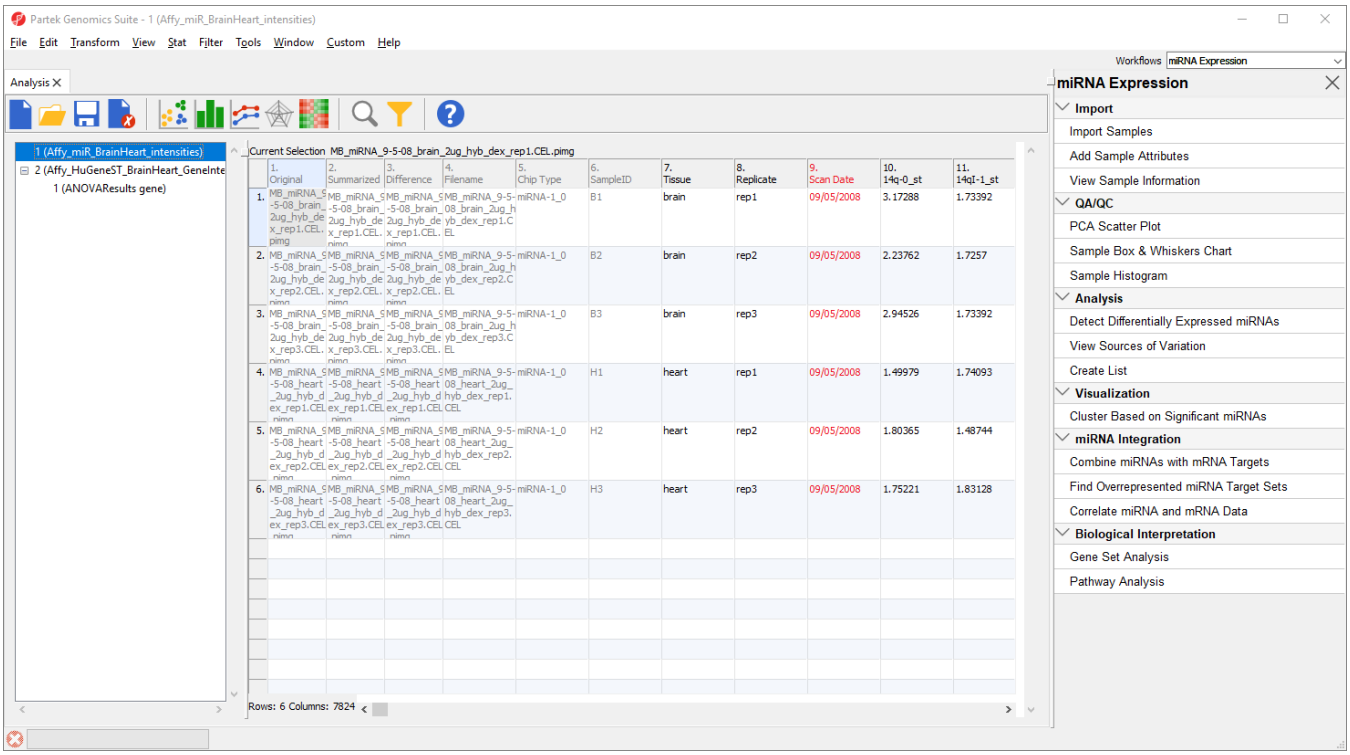


Figure 16. Viewing the miRNA probe intensities spreadsheet

- Select **PCA Scatter Plot** from the *QA/QC* section of the workflow

A new tab will open showing a PCA scatter plot (Figure 3).

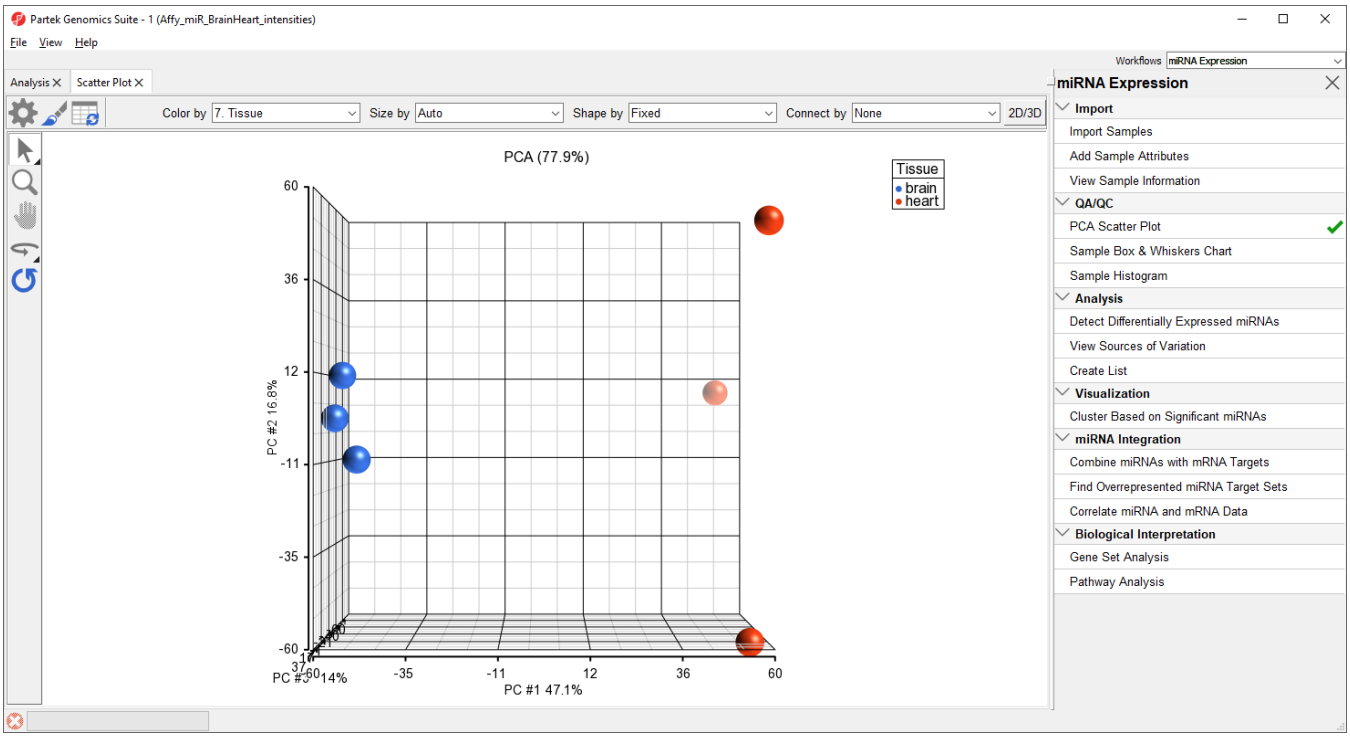


Figure 17. PCA scatter plot. Samples are spheres. Samples with more similar miRNA expression are close together while dissimilar samples are further apart.

In this PCA scatter plot, each point represents a sample in the spreadsheet. Points that are close together in the plot are more similar, while points that are far apart in the plot are more dissimilar.

To better view the data, we can rotate the plot.

- Select ( ) to activate *Rotate Mode*
- Click and drag to rotate the plot

Rotating the plot allows us to look for outliers in the data on each of the three principal components (PC1-3). The percentage of the total variation explained by each PC is listed by its axis label. The chart label shows the sum percentage of the total variation explained by the displayed PCs.

Here, we can see that the brain and heart samples are well separated across PC1, which is expected.

For more information about customizing the plot, please see Exploring the data set with PCA from the *Gene Expression with Batch Effect* tutorial.

Detecting differentially expressed miRNAs

Next, we will identify miRNAs that are differentially expressed between brain and heart tissues.

- Select the **Analysis** tab
- Select the **Affy_miR_BrainHeart_intensities** spreadsheet
- Select **Detect Differentially Expressed miRNAs** from the *Analysis* section of the workflow

The *ANOVA* dialog (Figure 4) allows us to configure the comparisons we want to make between samples and groups within the data set.

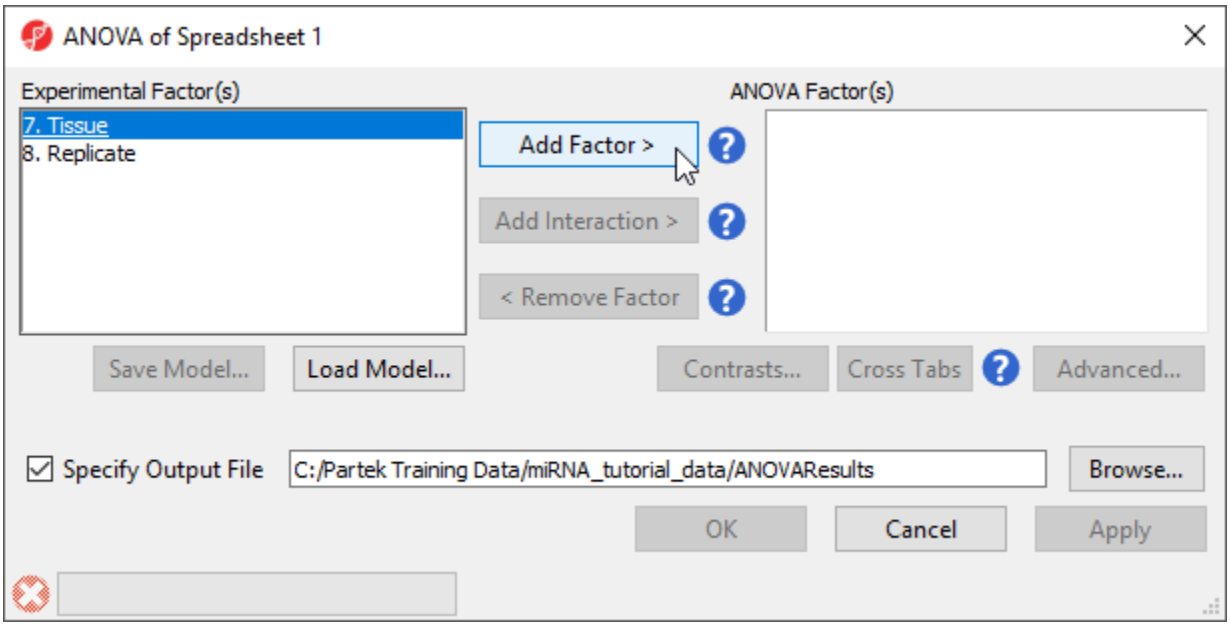


Figure 18. ANOVA dialog

- Select **Tissue** from the *Experimental Factor(s)* panel
- Select **Add Factor >** to move *Tissue* to the *ANOVA Factor(s)* panel

The *Contrasts...* button will now be available to select.

- Select **Contrasts...**

The *Configure ANOVA* dialog (Figure 5) is used to set up contrasts. Contrasts are the comparisons between groups and are where experimental questions can be asked. In this study, we are asking what miRNAs are differentially expressed between heart and brain tissue.

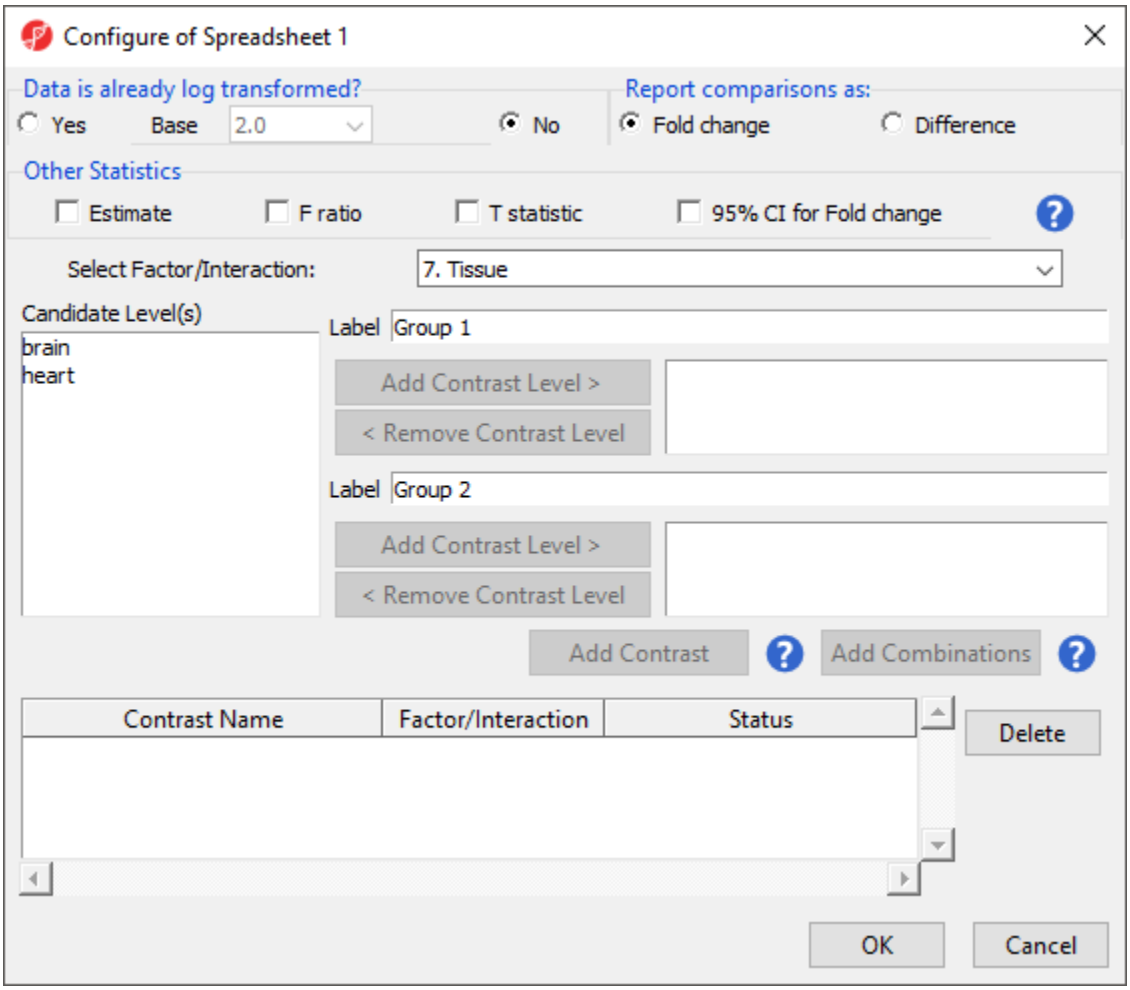


Figure 19. ANOVA configuration dialog

- Select **Yes** for *Data is already log transformed?*
- Select **Fold change** for *Report comparisons as*
- Select **7. Tissue** from the *Select Factor/Interaction* drop-down menu
- Select **brain** from the left panel
- Select **Add Contrast Level >** to move *brain* to the upper group - initially Group 1
- Select **heart** from the left panel

- Select **Add Contrast Level >** to move *heart* to the lower group - initially Group 2

This contrast (Figure 6) will compare expression of miRNAs in brain samples to expression in heart samples with brain as the numerator and heart as the denominator for fold-change calculations.

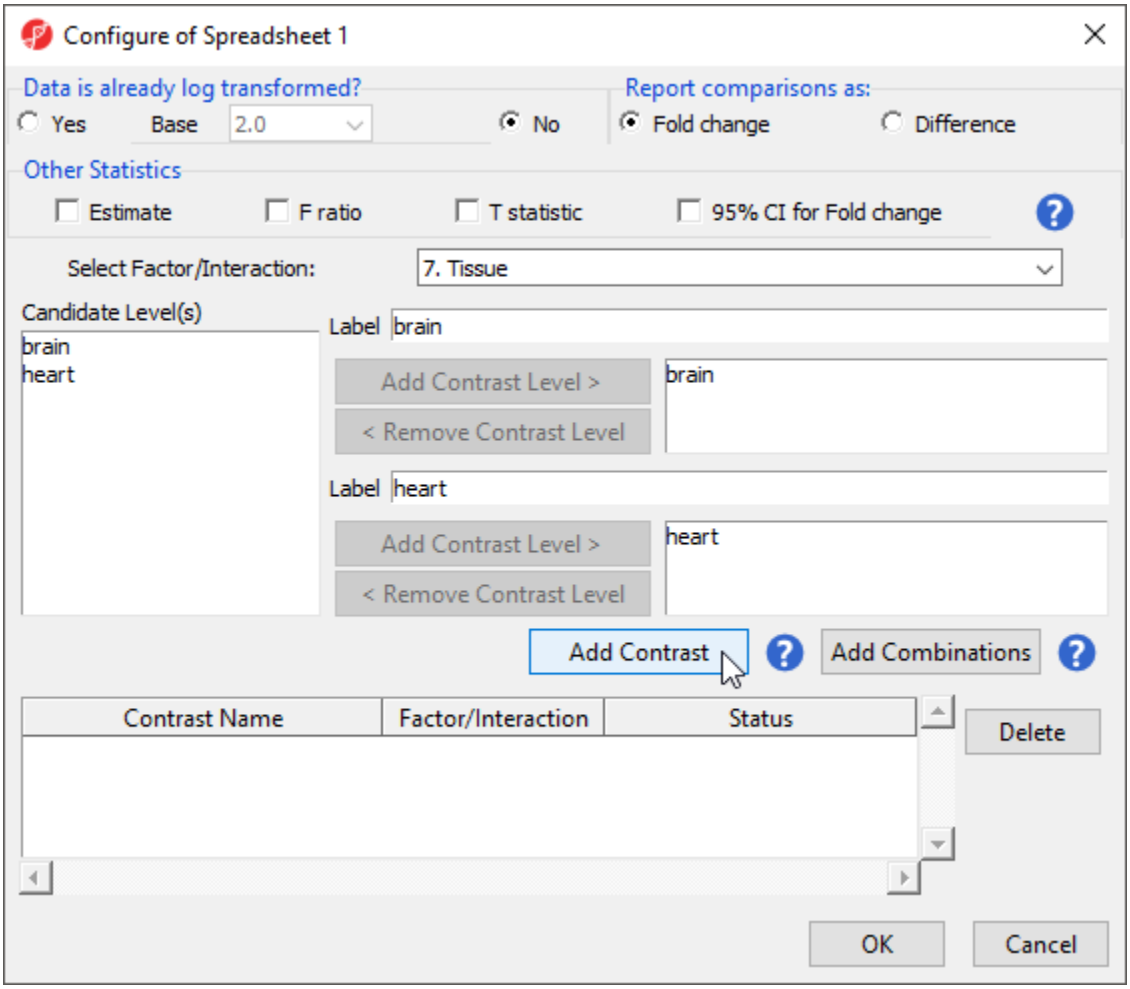


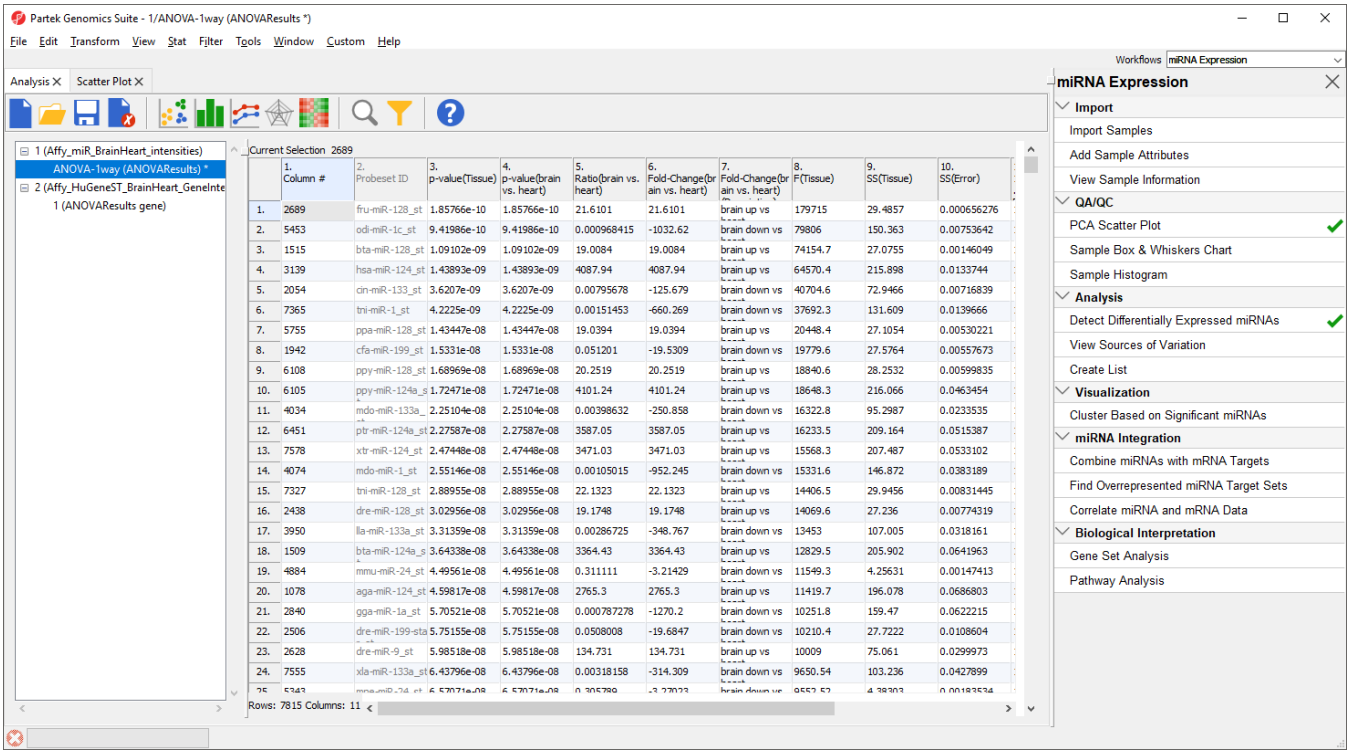
Figure 20. Configuring a contrast between brain and heart tissue in the ANOVA dialog

- Select **Add Contrast**
- Select **OK**

The *Contrasts...* button should now read *Contrasts Included.*

- Select **OK** to run the ANOVA as configured

An ANOVA Results sheet, *ANOVAResults*, will be created as a child spreadsheet of *Affy_miR_BrainHeart_intensities* (Figure 7). In this spreadsheet, each row represents a probe set and the columns represent the computation results for that probe set. Although not synonymous, probe set and gene will be treated as synonyms in this tutorial for convenience. By default, the genes are sorted in ascending order by the p-value of the first categorical factor, which, in this case, is *Tissue*. This means the most significant differentially expressed miRNAs between the brain and heart (up-regulated and donw-regulated) are at the top of the spreadsheet.



	1. Column #	2. Probeset ID	3. p-value(Tissue)	4. p-value(brain vs. heart)	5. Ratio(brain vs. heart)	6. Fold-Change(brain vs. heart)	7. Fold-Change(brain vs. heart)	8. F(Tissue)	9. SS(Tissue)	10. SS(Error)
1.	2689	fru-miR-128_st	1.85766e-10	1.85766e-10	21.6101	21.6101	brain up vs	179715	29.4857	0.000656276
2.	5453	odi-miR-1c_st	9.41986e-10	9.41986e-10	0.000968415	-1032.62	brain down vs	79806	150.363	0.00753642
3.	1515	bta-miR-128_st	1.09102e-09	1.09102e-09	19.0084	19.0084	brain up vs	74154.7	27.0755	0.00146049
4.	3139	hsa-miR-124_st	1.43893e-09	1.43893e-09	4087.94	4087.94	brain up vs	64570.4	215.898	0.0133744
5.	2054	cin-miR-133_st	3.6207e-09	3.6207e-09	0.00795678	-125.679	brain down vs	40704.6	72.9466	0.00716839
6.	7365	tni-miR-1_st	4.2225e-09	4.2225e-09	0.00151453	-660.269	brain down vs	37692.3	131.609	0.0139666
7.	5755	ppa-miR-128_st	1.43447e-08	1.43447e-08	19.0394	19.0394	brain up vs	20448.4	27.1054	0.00530221
8.	1942	cfa-miR-199_st	1.5331e-08	1.5331e-08	0.051201	-19.5309	brain down vs	19779.6	27.5764	0.00557673
9.	6108	ppy-miR-128_st	1.68969e-08	1.68969e-08	20.2519	20.2519	brain up vs	18840.6	28.2532	0.00599835
10.	6105	ppy-miR-124a_s	1.72471e-08	1.72471e-08	4101.24	4101.24	brain up vs	18648.3	216.066	0.0463454
11.	4034	mdo-miR-133a_	2.25104e-08	2.25104e-08	0.00398632	-250.858	brain down vs	16322.8	95.2987	0.0233535
12.	6451	ptr-miR-124a_st	2.27587e-08	2.27587e-08	3587.05	3587.05	brain up vs	16233.5	209.164	0.0515387
13.	7578	xtr-miR-124_st	2.47448e-08	2.47448e-08	3471.03	3471.03	brain up vs	15568.3	207.487	0.0533102
14.	4074	mdo-miR-1_st	2.55146e-08	2.55146e-08	0.00105015	-952.245	brain down vs	15331.6	146.872	0.0383189
15.	7327	tni-miR-128_st	2.88955e-08	2.88955e-08	22.1323	22.1323	brain up vs	14406.5	29.9456	0.00831445
16.	2438	dre-miR-128_st	3.02956e-08	3.02956e-08	19.1748	19.1748	brain up vs	14069.6	27.236	0.00774319
17.	3950	lla-miR-133a_st	3.31359e-08	3.31359e-08	0.00286725	-348.767	brain down vs	13453	107.005	0.0318161
18.	1509	bta-miR-124a_s	3.64338e-08	3.64338e-08	3364.43	3364.43	brain up vs	12829.5	205.902	0.0641963
19.	4884	mmu-miR-24_st	4.49561e-08	4.49561e-08	0.311111	-3.21429	brain down vs	11549.3	4.25631	0.00147413
20.	1078	aga-miR-124_st	4.59817e-08	4.59817e-08	2765.3	2765.3	brain up vs	11419.7	196.078	0.0686803
21.	2840	gga-miR-1a_st	5.70521e-08	5.70521e-08	0.000787278	-1270.2	brain down vs	10251.8	159.47	0.0622215
22.	2506	dre-miR-199-sta	5.75155e-08	5.75155e-08	0.0508008	-19.6847	brain down vs	10210.4	27.7222	0.0108604
23.	2628	dre-miR-9_st	5.98518e-08	5.98518e-08	134.731	134.731	brain up vs	10009	75.061	0.0299973
24.	7555	xla-miR-133a_st	6.43796e-08	6.43796e-08	0.00318158	-314.309	brain down vs	9650.54	103.236	0.0427899

Figure 21. Viewing the ANOVA results spreadsheet

You may explore what is known about any listed miRNA using external databases TargetScan, miRBase, microRNA.org, or miR2Disease, by right-clicking a row header, selecting *Find miRNA in...* and choosing one of the external databases. This will open a web page in your default web browser and requires your computer be connected to the internet.

For more information about AVOVA in Partek Genomics Suite, see Identifying differentially expressed genes using ANOVA.

Creating a list of miRNAs of interest

The ANOVA results spreadsheet includes every miRNA on the array for a total of 7815 miRNAs. However, many of these miRNAs are not significantly differentially expressed between brain and heart and, thus, are not of interest. Next, we will create a filtered list of significantly differentially expressed miRNAs.

- Select the **ANOVAResults** spreadsheet
- Select **Create List** from the *Analysis* section of the workflow

The *List Manager* dialog will open (Figure 8).

- Select **brain vs. heart** under *Contrast: find genes that change between two categories*

By default, the fold-change and significance thresholds are set to > 2, < -2 and p-value with FDR < 0.05. These defaults are appropriate for this tutorial so we will leave them in place.

- Select **Create** to create a new list, *brain vs. heart* containing only the 1404 miRNAs that pass the criteria

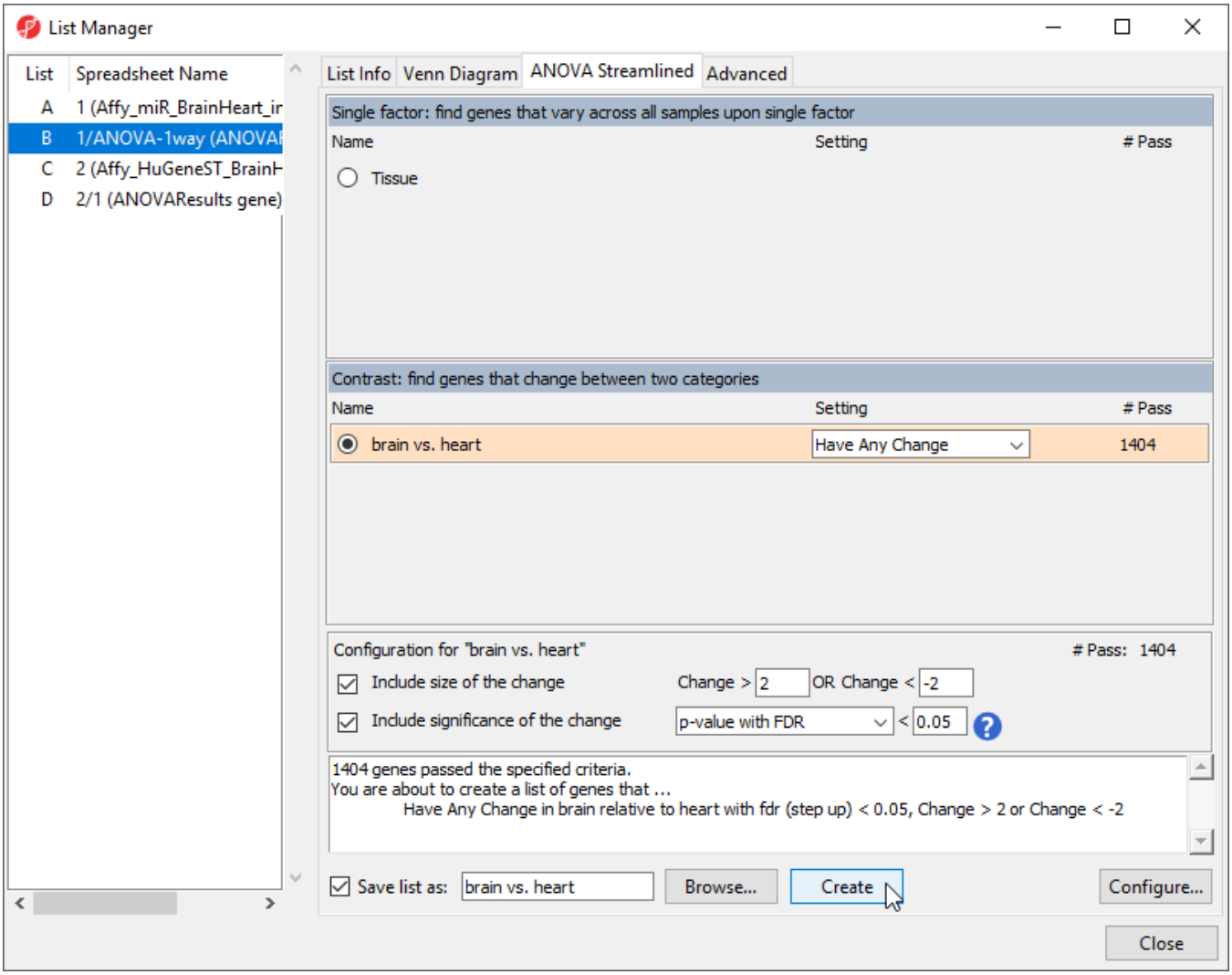


Figure 22. Creating a list of significantly differentially expressed miRNAs

A new spreadsheet, *brain vs. heart* will be created as a child spreadsheet of *Affy_miR_BrainHeart* (Figure 9).

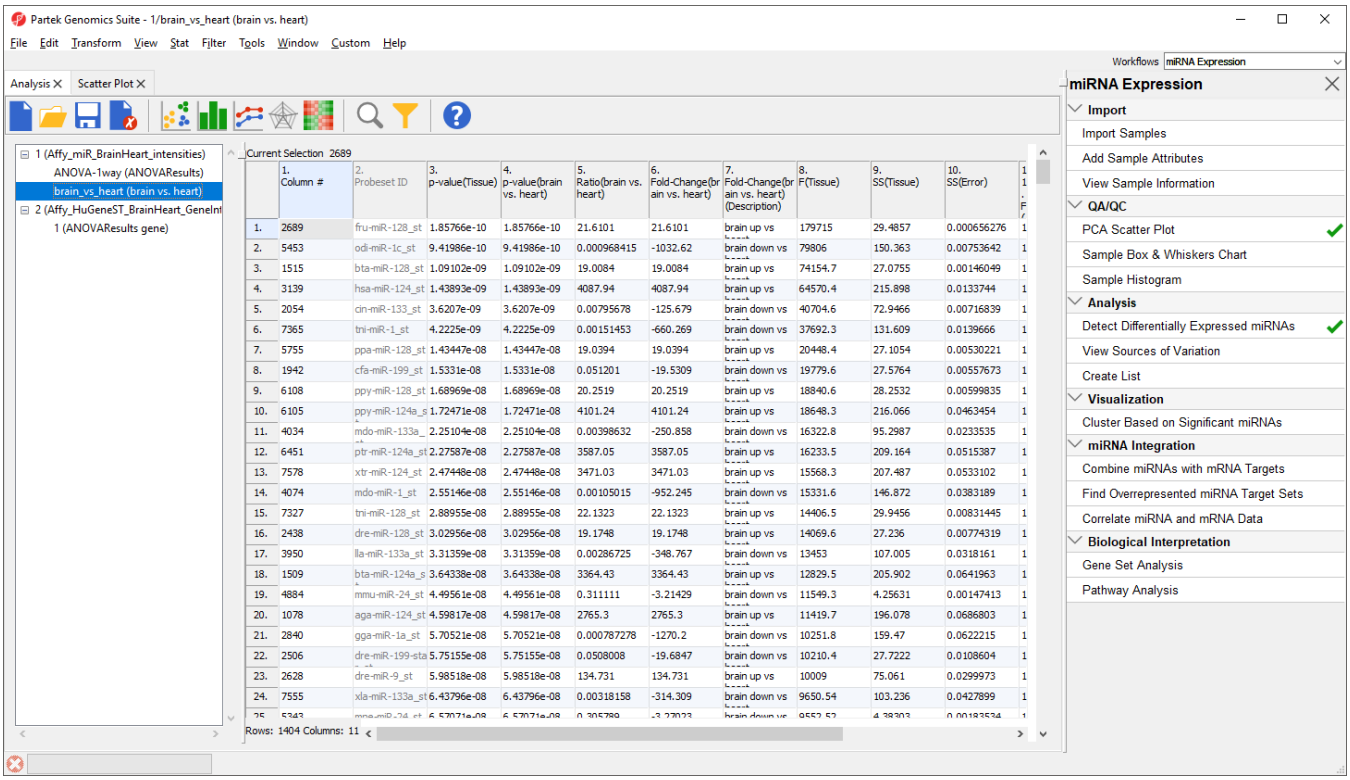


Figure 23. Viewing brain vs. heart spreadsheet

To view the miRNAs with the largest difference between tissues, we can sort by fold-change.

- Right-click the *6. Fold-Change(brain vs. heart)* column header
- Select **Sort Descending by Absolute Value** from the pop-up menu

The top 33 miRNAs we see (Figure 10) are all miR-124 from different species. The miRNA miR-124 is the most abundant miRNA in neuronal cells so this finding is expected. The multiple species versions of miR-124 are present because Affymetrix GeneChip miRNA arrays provide comprehensive coverage of miRNAs from multiple organisms including human, mouse, rat, canine, monkey, and many more on a single chip. The miRNAs from these different species are highly homologous so probes targeting miRNAs from other species will hybridize with human miRNAs. Therefore, we need to filter the list of miRNAs to include only human miRNAs.

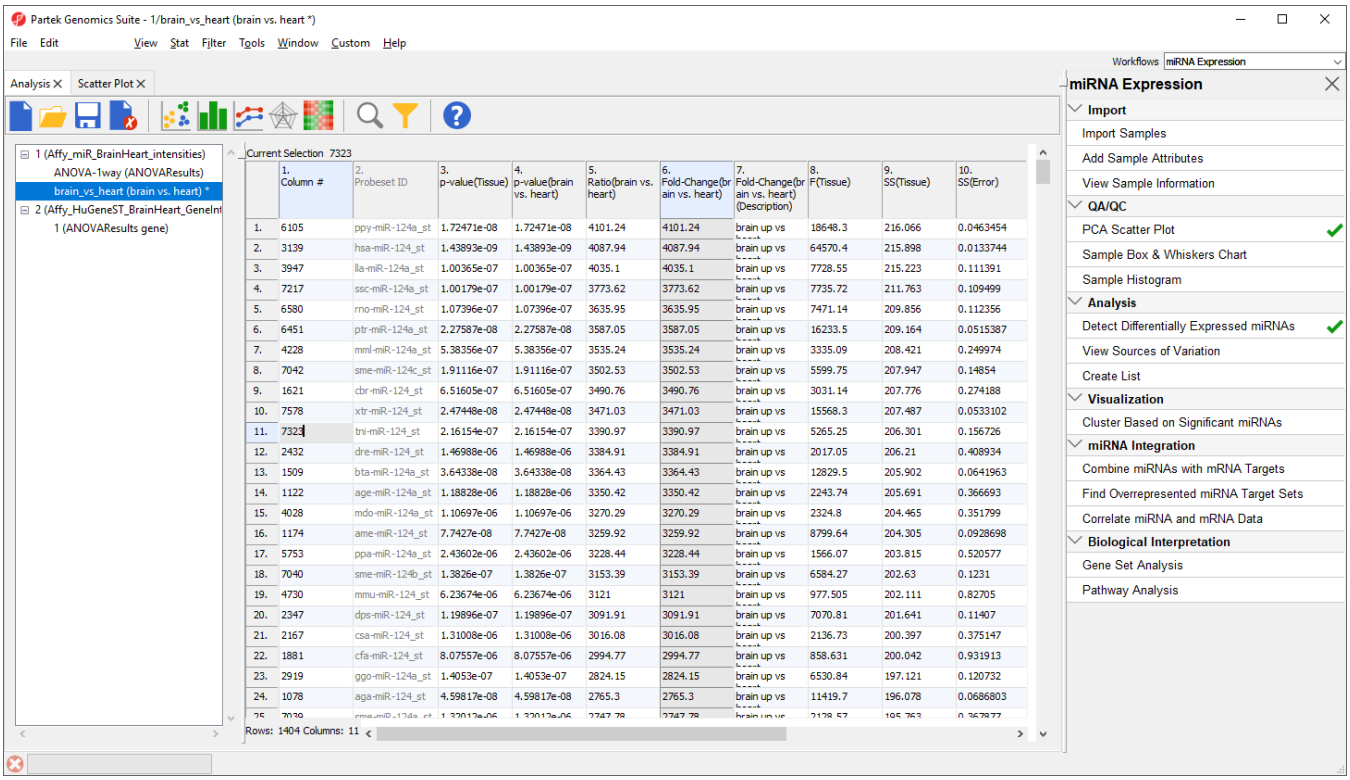


Figure 24. miR-124 is highly differentially expressed in brain vs. heart

To do this, we need to add a new annotation column containing species information for each probe.

- Right-click on the *2. Probeset ID* column header
- Select **Insert Annotation** from the pop-up menu
- Select **Add as categorical**
- Check **Species Scientific Name** (Figure 11)
- Select **OK** to add the annotation column

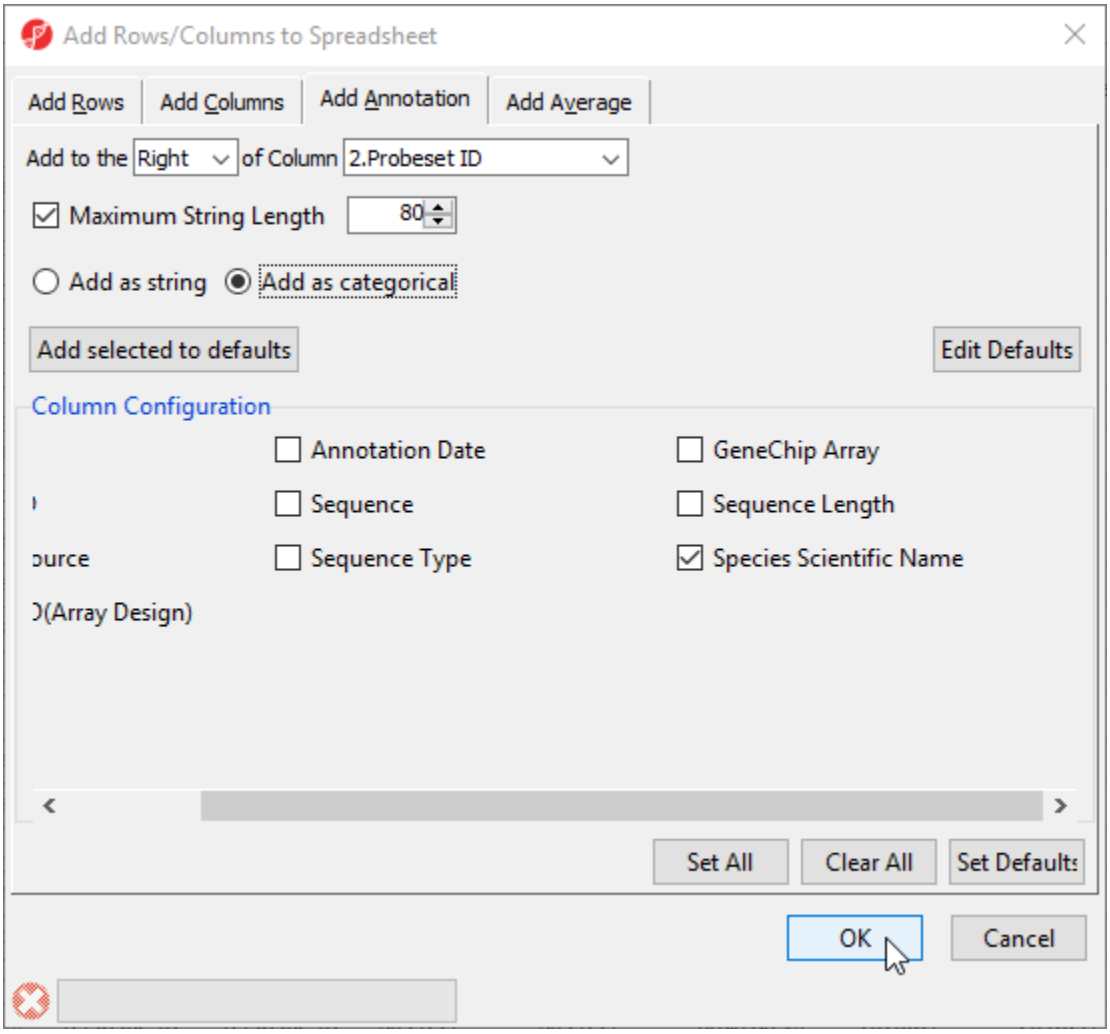


Figure 25. Inserting species annotation column

The table now includes a column *3. Species Scientific Name* with the species name of each miRNA. We can now filter to include only human miRNAs.

- Right-click the *3. Species Scientific Name* column header
- Select **Find / Replace / Select...** from the pop-up menu
- Type **Homo sapiens** for *Find What*
- Select **Only in column** for *Search*

- Select **3. Species Scientific Name** from the drop-down menu next to the *Only in column* option
- Select **Select All** (Figure 12)

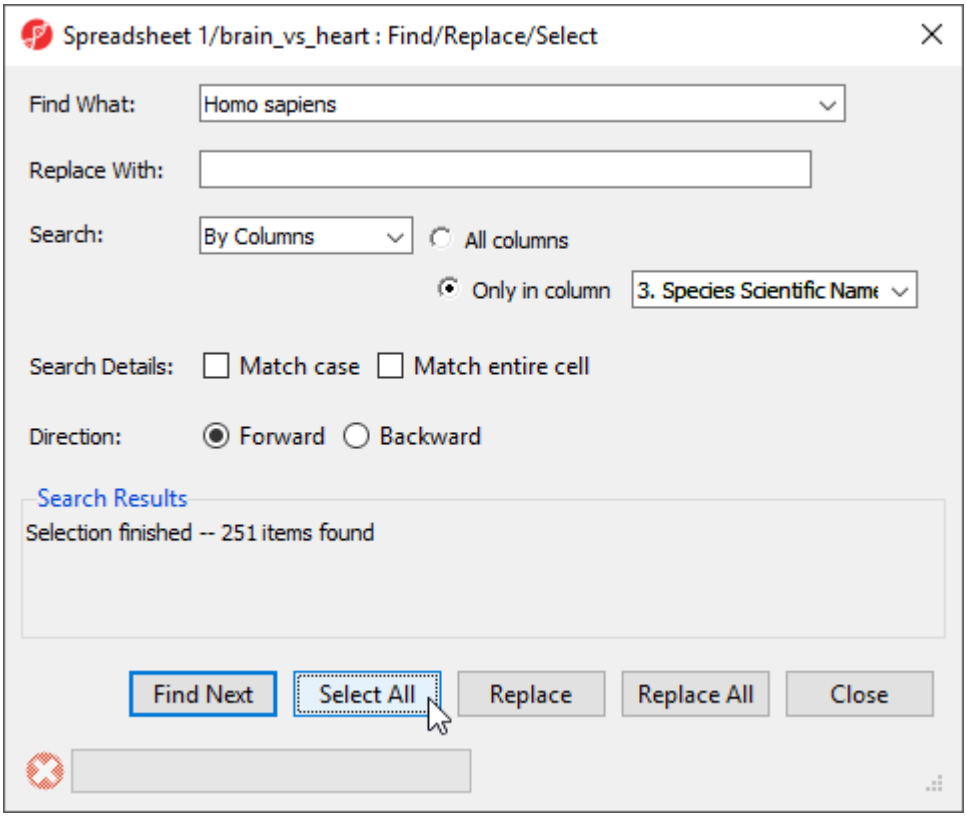


Figure 26. Configuring the Find / / Replace / Select... dialog

The search should find and select 251 miRNAs.

- Select **Close**
- Right-click any of the row headers that are selected
- Select **Filter Include** from the pop-up menu

The spreadsheet will now include only the 251 miRNAs from human (Figure 13). The first row is still miR-124 with a fold change of 4087.94. The black and gold bar on the right-hand side of the spreadsheet indicates the fraction of rows that have been filtered. To retain this filtered list, we can create a new spreadsheet.

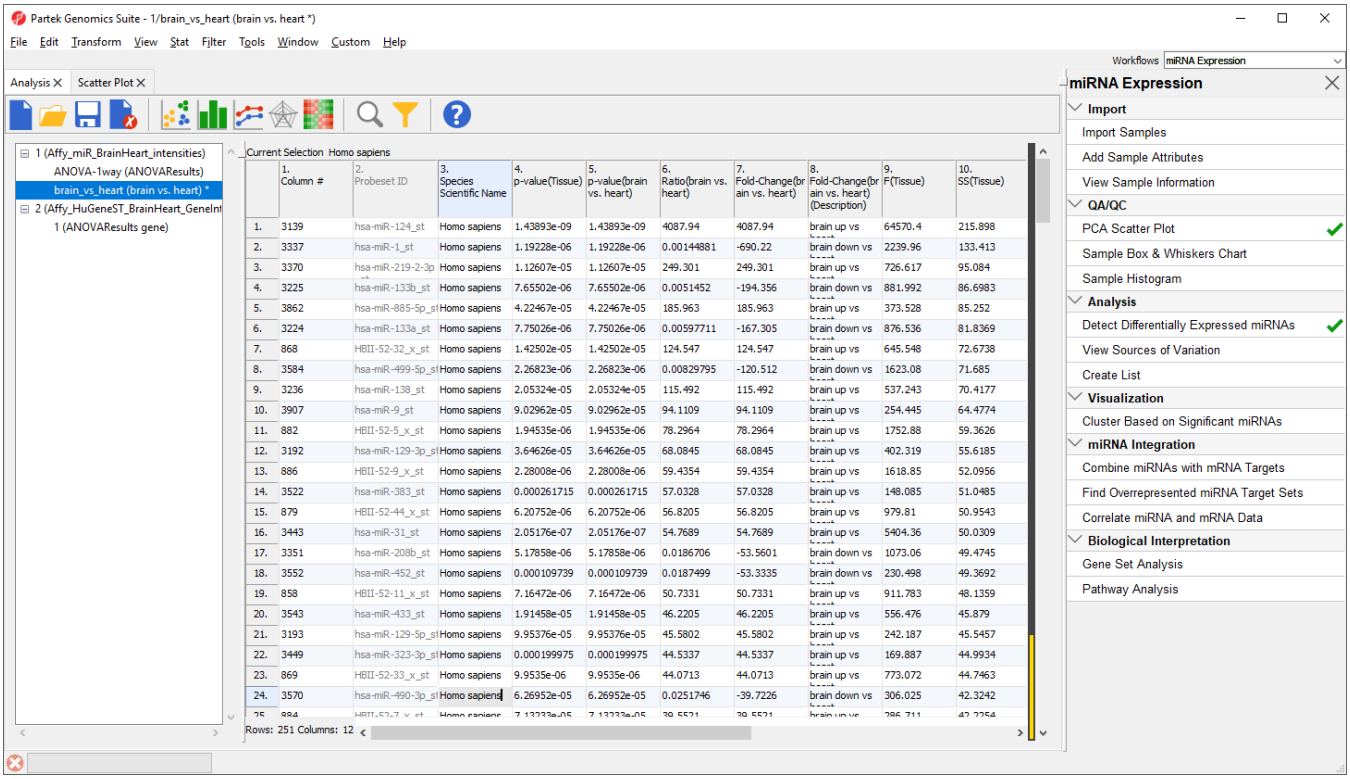


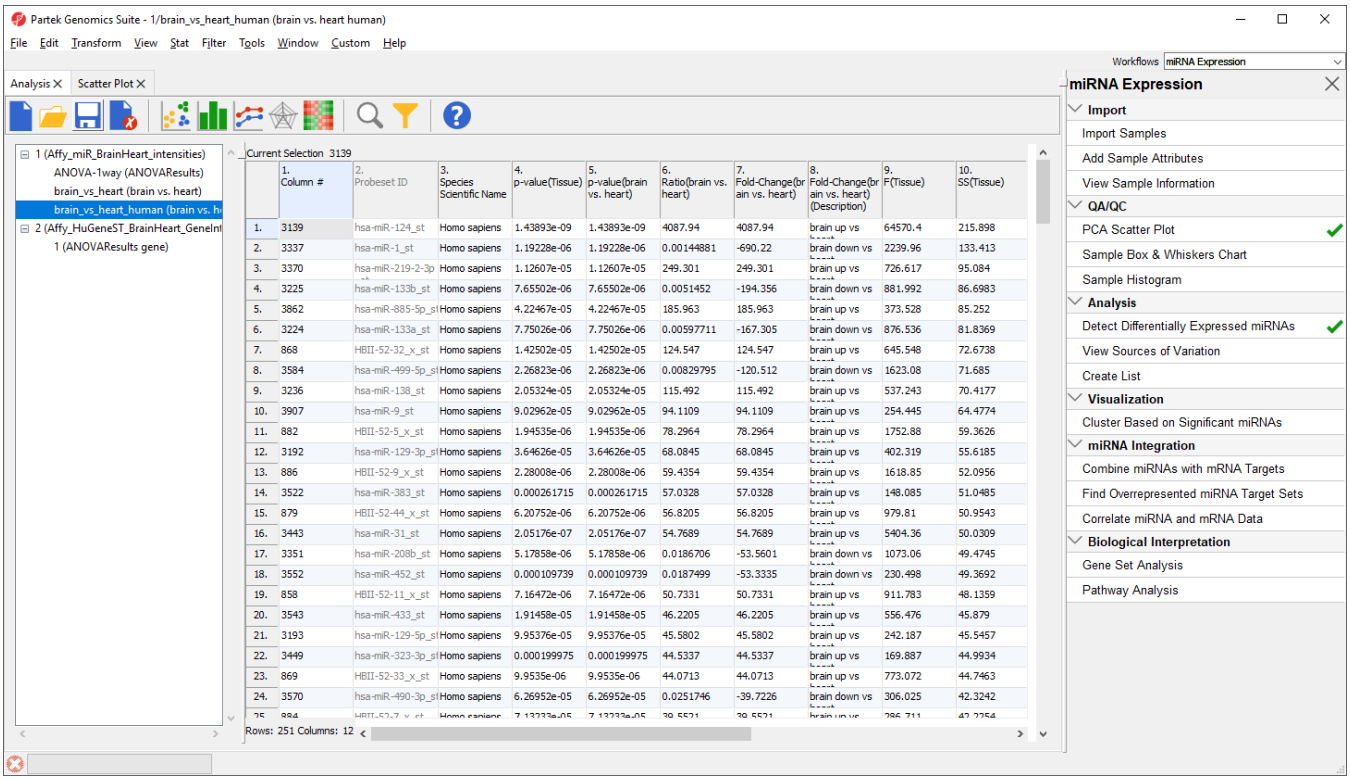
Figure 27. Viewing differentially expressed human miRNAs

- Right-click the *brain_vs_heart* spreadsheet in the spreadsheet tree
- Select **Clone...** from the pop-up menu

Cloning a spreadsheet while a filter is applied copies only the included rows/columns.

- Name the spreadsheet **brain_vs_heart_human**
- Select **Affy_miR_BrainHeart_intensities** from the drop-down menu *Create new spreadsheet as a child of spreadsheet*
- Select
- Name the new file **brain vs. heart human**
- Select **Save**

The new spreadsheet includes only the 251 human miRNAs that are significantly differentially expressed between brain and heart tissue (Figure 14).



	1. Column #	2. Probeset ID	3. Species Scientific Name	4. p-value(Tissue)	5. p-value(brain vs. heart)	6. Ratio(brain vs. heart)	7. Fold-Change(brain vs. heart)	8. Fold-Change(brain vs. heart) (Description)	9. F(Tissue)	10. SS(Tissue)
1.	3139	hsa-miR-124_st	Homo sapiens	1.43893e-09	1.43893e-09	4087.94	4087.94	brain up vs	64570.4	215.898
2.	3337	hsa-miR-1_st	Homo sapiens	1.19228e-06	1.19228e-06	0.00144881	-690.22	brain down vs	2239.96	133.413
3.	3370	hsa-miR-219-2-3p	Homo sapiens	1.12607e-05	1.12607e-05	249.301	249.301	brain up vs	726.617	95.084
4.	3225	hsa-miR-133b_st	Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983
5.	3862	hsa-miR-885-5p_st	Homo sapiens	4.22467e-05	4.22467e-05	185.963	185.963	brain up vs	373.528	85.252
6.	3224	hsa-miR-133a_st	Homo sapiens	7.75026e-06	7.75026e-06	0.00597711	-167.305	brain down vs	876.536	81.8369
7.	868	HBII-52-32_x_st	Homo sapiens	1.42502e-05	1.42502e-05	124.547	124.547	brain up vs	645.548	72.6738
8.	3584	hsa-miR-499-5p_st	Homo sapiens	2.26823e-06	2.26823e-06	0.00829795	-120.512	brain down vs	1623.08	71.685
9.	3236	hsa-miR-138_st	Homo sapiens	2.05324e-05	2.05324e-05	115.492	115.492	brain up vs	537.243	70.4177
10.	3907	hsa-miR-9_st	Homo sapiens	9.02962e-05	9.02962e-05	94.1109	94.1109	brain up vs	254.445	64.4774
11.	882	HBII-52-5_x_st	Homo sapiens	1.94535e-06	1.94535e-06	78.2964	78.2964	brain up vs	1752.88	59.3626
12.	3192	hsa-miR-129-3p_st	Homo sapiens	3.64626e-05	3.64626e-05	68.0845	68.0845	brain up vs	402.319	55.6185
13.	886	HBII-52-9_x_st	Homo sapiens	2.28008e-06	2.28008e-06	59.4354	59.4354	brain up vs	1618.85	52.0956
14.	3522	hsa-miR-383_st	Homo sapiens	0.000261715	0.000261715	57.0328	57.0328	brain up vs	148.085	51.0485
15.	879	HBII-52-44_x_st	Homo sapiens	6.20752e-06	6.20752e-06	56.8205	56.8205	brain up vs	979.81	50.9543
16.	3443	hsa-miR-31_st	Homo sapiens	2.05176e-07	2.05176e-07	54.7689	54.7689	brain up vs	5404.36	50.0309
17.	3351	hsa-miR-208b_st	Homo sapiens	5.17858e-06	5.17858e-06	0.0186706	-53.5601	brain down vs	1073.06	49.4745
18.	3552	hsa-miR-452_st	Homo sapiens	0.000109739	0.000109739	0.0187499	-53.3335	brain down vs	230.498	49.3692
19.	858	HBII-52-11_x_st	Homo sapiens	7.16472e-06	7.16472e-06	50.7331	50.7331	brain up vs	911.783	48.1359
20.	3543	hsa-miR-433_st	Homo sapiens	1.91458e-05	1.91458e-05	46.2205	46.2205	brain up vs	556.476	45.879
21.	3193	hsa-miR-129-5p_st	Homo sapiens	9.95376e-05	9.95376e-05	45.5802	45.5802	brain up vs	242.187	45.5457
22.	3449	hsa-miR-323-3p_st	Homo sapiens	0.000199975	0.000199975	44.5337	44.5337	brain up vs	169.887	44.9934
23.	869	HBII-52-33_x_st	Homo sapiens	9.9535e-06	9.9535e-06	44.0713	44.0713	brain up vs	773.072	44.7463
24.	3570	hsa-miR-490-3p_st	Homo sapiens	6.26952e-05	6.26952e-05	0.0251746	-39.7226	brain down vs	306.025	42.3242
25.	884	HBII-52-7_x_st	Homo sapiens	7.13233e-05	7.13233e-05	39.5521	39.5521	brain up vs	286.711	42.2254

Figure 28. Viewing the filtered human miRNAs spreadsheet

The next step in our analysis will be integrating miRNA and gene expression data.

« miRNA Expression and Integration with Gene Expression Integrate miRNA and Gene Expression data »

Additional Assistance

If you need additional assistance, please visit our support page to submit a help ticket or find phone numbers for regional support.

Your Rating: ☆☆☆☆☆ Results: ★★★★★ 32 rates