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).

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VOVAResults gene)		Column #	Probeset ib	nt	Gene Symbol	Kerbeg	p-value(rissue)	e)	vs. Heart)	Heart)	ain vs. Heart)	4	View Sample Information
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	1.	18661	8083779	NM_001122752	2 SERPINI1	NM_001122752	2.80113e-06	0.00048932	2.80113e-06	34.1381	34.1381		PCA Scatter Plot
	2.	24797	8143028	NM_017812 //	CHCHD3	NM_017812	2.99382e-06	0.000526954	2.99382e-06	0.196247	-5.09563		Sample Box & Whiskers Chart
	3.	22014	8116534	NM_032765 //	TRIM52	NM_032765	3.42305e-06	2.08268-05	3.42305e-06	1.86134	1.86134		Sample Histogram
	4.	28050	8172858	NM_001111125	IQSEC2	NM_001111125	7.90025e-06	0.000870654	7.90025e-06	3.19735	3.19735		
	5.	15/53	8054166	NM_025244 //	ISGA 10	NM_025244	7.94332e-06	0.000130049	7.94332e-06	2.00348	2.00348		Detect Differentially Expressed miRNA
	6.	5829	7954021	NM_001310 //	CREBL2	NM_001310	8.70452e-06	3.38042e-05	8.70452e-06	2.07/51	2.07/51		Detect Differentially Expressed military
	1.	/612	7972650	NM_1/5929 //	FGF14	NM_175929	9.724312-06	0.0205134	9.724318-06	12.3537	12.3537		View Sources of Variation
	8.	6/55	7953851	INM_001098815	KIAAU748	INM_001098815	9.738828-06	0.0134063	9.738828-06	27.6273	27.6273		Create List
	9.	3597	7933071		79.07.4794		1.134/5e-05	2.40501e-05	1.134/50-05	1.59436	1.59436		\sim Visualization
	10.	13457	8031650	NM_020813 //	ZNF471	NM_020813	1.226628-05	0.000204846	1.226628-05	1.996/1	1.99671		Cluster Based on Significant miRNAs
	11.	12970	0020920	MACTO //	MASTS C11E70	DC0000001	1.547502-05	0.00540722	1.547508-05	11.9000	11.9000		✓ miRNA Integration
	12.	12154	7940400	DL002331 //		DC002551	1.000098-00	0.000279085	1.000098-00	0.697706	-1.45527		Combine miRNAs with mRNA Targets
	10.	14912	0020703	11 TTABATO //	00010	NM_000001303	2.146210.05	0.002/8082	2.146210.05	25 1074	25 1074		Find Querranted wiDNA Travel Q
	15	10462	8001325	DDD10 //	DFF 10	1441_020868	2.140312403	0.000547233	2.110310-03	0.006300	-1 10327		Find Overrepresented miRNA Target Se
	16	11517	9012423	NM 025000 //	C17orf69	NM 025000	2.217010-03	0.000129607	2.2170-12-05	1 74602	1 74602		Correlate miRNA and mRNA Data
	17	20625	9102260	NM 017620 //	DCHS2	NM 017620	2.243776-03	0.000129007	2.245776-05	2 90252	2 90252		✓ Biological Interpretation
	18	12702	8024003	NM 002579 //	PALM	NM 002579	2 28249e-05	0.00878101	2 28249e-05	2 04898	2 04898		Gene Set Analysis
	19.	25212	8146839	BC041961 //	C8orf34	BC041961	2.53926e-05	0.102525	2.53926e-05	2.56046	2.56046		Pathway Analysis
	20.	11576	8013094	NM 003653 //	COPS3	NM 003653	2.92404e-05	0.00152943	2.92404e-05	0.661699	-1.51126		· · ·
	21.	13592	8032899	NM 182919 //	TICAM1	NM 182919	2.94448e-05	5.33176e-05	2.94448e-05	0.846523	-1.1813		
	22.	7636	7972810	NM 024537 //	CARS2	NM 024537	3.04141e-05	0.00228575	3.04141e-05	0.481994	-2.07471		
	23.	6595	7962146	NM 021238 //	EAM60A	NM 021238	3.08522e-05	0.000419985	3.08522e-05	0.225215	-4.44021		
	24.	22949	8124942	NM 130463 //	ATP6V1G2	NM 130463	3.31027e-05	0.0262198	3.31027e-05	27,7308	27,7308		
V	Power	78869 Columna	- 14			1.5.1_200.100				2.17000			

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 is a 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.

Partek Genomics Suite - 1 (Affy_miR_BrainHe	eart_intensities)											- 🗆 X
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1 (Affy_miR_BrainHeart_intensities)	Current Selection	MB_mIRNA_	9-5-08_brain_2ug_hyb_dex 3. 4.		6.	7.	8.	9.	10.	11.	· ^	Add Sample Attributes
1 (ANOVAResults gene)	Original	Summarized	Difference Filename	Chip Type	SampleID	Tissue	Replicate	Scan Date	14q-0_st	14qI-1_st		View Sample Information
	15-08_brain_	MB_miRNA_9 -5-08_brain_	MB_miRNA_9MB_miRNA_9 -5-08_brain_08_brain_2ug	-5- miRNA-1_0 j_h	B1	brain	rep1	09/05/2008	3.17288	1.73392		∼ qa/qc
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	2. MB_miRNA_9	MB_miRNA_9	MB_miRNA_9MB_miRNA_9	-5- miRNA-1_0	B2	brain	rep2	09/05/2008	2.23762	1.7257		Sample Box & Whiskers Chart
	-5-08_brain_ 2ug_hyb_de	2ug_hyb_de	2ug_hyb_de_yb_dex_rep2	.C								Sample Histogram
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	3. MB_miRNA_9 -5-08_brain_	MB_miRNA_9 -5-08_brain_	MB_miRNA_9MB_miRNA_9 -5-08_brain_08_brain_2ug	-5- miRNA-1_0 j_h	B3	brain	rep3	09/05/2008	2.94526	1.73392		Detect Differentially Expressed miRNAs
	2ug_hyb_de x_rep3.CEL.	2ug_hyb_de x_rep3.CEL.	2ug_hyb_de yb_dex_rep3 x_rep3.CEL. EL	.C								View Sources of Variation
	4. MB_miRNA_9	MB_miRNA_9	MB_miRNA_9MB_miRNA_9	-5- miRNA-1_0	H1	heart	rep1	09/05/2008	1.49979	1.74093		Create List
	-5-08_heart _2ug_hyb_d	-5-08_heart _2ug_hyb_d	-5-08_heart_08_heart_2u _2ug_hyb_d hyb_dex_rep	7 1.								argee Visualization
	ex_rep1.CEL	ex_rep1.CEL	.ex_rep1.CELCEL 					(Cluster Based on Significant miRNAs
	5. MB_mRNA_9 -5-08_heart	-5-08_heart	-5-08_heart_08_heart_2u	-5-mRNA-1_0	H2	heart	rep2	09/05/2008	1.80365	1.48/44		\sim miRNA Integration
	_2ug_hyb_d ex_rep2.CEL	_2ug_hyb_d ex_rep2.CEL	2ug_hyb_d hyb_dex_rep .ex_rep2.CEL CEL	2.								Combine miRNAs with mRNA Targets
	6. MB_miRNA_9	MB_miRNA_9	MB_miRNA_9MB_miRNA_9	-5- miRNA-1_0	H3	heart	rep3	09/05/2008	1.75221	1.83128		Find Overrepresented miRNA Target Sets
	-5-08_heart _2ug_hyb_d	-5-08_heart _2ug_hyb_d	-5-08_heart_08_heart_2u _2ug_hyb_d_hyb_dex_rep	7 3.								Correlate miRNA and mRNA Data
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	-											Gene Set Analysis
	-											Pathway Analysis
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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

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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.

ANOVA of Spreadsheet 1	×
Experimental Factor(s) 7. Tissue 8. Replicate	ANOVA Factor(s) Add Factor > Add Interaction > < Remove Factor
Save Model Load Model	Contrasts Cross Tabs 🕜 Advanced
Specify Output File C:/Partek Trainin	ng Data/miRNA_tutorial_data/ANOVAResults Browse
	OK Cancel Apply

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.

Configure of Spreadshe	et 1				×
Data is already log transform Yes Base 2.0 Others Statistics	med?	⊙ No	Report compa Fold change	orisons as: C Diffe	rence
Estimate F	ratio	T statistic	95% 0	CI for Fold change	0
Select Factor/Interaction Candidate Level(s)	Label	7. Tissue Group 1			~
brain heart	A < R	dd Contrast Level > emove Contrast Lev	/el		
	Label	Group 2			
	< R	emove Contrast Level >	/el		
		Ad	d Contrast	? Add Combin	ations ?
Contrast Name		Factor/Interaction	Stat	us	Delete
				–	
•				OK	Cancel

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.

Configure of Spreadshee	et 1				×
Data is already log transform O Yes Base 2.0	ned?	No R	eport compariso Fold change	ns as: C Differen	nce
Estimate F	ratio 🗌 T si	atistic	95% CI fo	r Fold change	0
Select Factor/Interaction: Candidate Level(s)	7. Tissue				~
brain heart	Add Contrast < Remove Con	Level >	brain		
	Label heart				
	Add Contrast	Level > trast Level	heart		
		Add Co	ontrast 💦 🕜	Add Combinat	tions ?
Contrast Name	Factor/Inter	action	Status		Delete
4				▼	
				ОК	Cancel

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.

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	_ V	Y -	\sim	•									Import Samples	
□ 1 (Affy_miR_BrainHeart_intensities)	Curren	t Selection 268	•									^	Add Sample Attributes	
ANOVA-1way (ANOVAResults) *		1. Column #	2. Probeset ID	 p-value(Tissue) 	4. p-value(brain	5. Ratio(brain vs.	6. Fold-Change(br	7. Fold-Change(br	8. F(Tissue)	9. SS(Tissue)	10. SS(Error)		View Sample Information	
2 (Affy_HuGeneST_BrainHeart_GeneInte 1 (ANO)(AD)					vs. heart)	heart)	ain vs. heart)	ain vs. heart)				12		
I (ANOVAResults gene)	1.	2689	fru-miR-128_st	1.85766e-10	1.85766e-10	21.6101	21.6101	brain up vs	179715	29.4857	0.000656276		DCA Sector Plat	
	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		Sample Box & Whiskers Chart	
	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		Sample Histogram	
	5.	2054	an-mR-133_st	3.620/e-09	3.6207e-09	0.00/956/8	-125.679	brain down vs	40704.6	72.9466	0.00716839		✓ Analysis	
	6.	7365	tni-mR-1_st	4.2225e-09	4.2225e-09	0.00151453	-660.269	brain down vs	37692.3	131.609	0.0139666		Detect Differentially Expressed miRNAs	-
	1.	5/55	ppa-mik-128_st	1.4344/e-08	1.4344/e-08	19.0394	19.0394	brain up vs	20448.4	27.1054	0.00530221		View Sources of Variation	
	8.	1942	cra-miR-199_st	1.53310-08	1.53310-08	0.051201	-19.5309	brain down vs	19779.6	27.5764	0.0055/6/3		Create List	
	9.	6105	ppy-mik-126_st	1.009090-00	1.009090-00	4101.24	20.2519	brain up vs	10040.0	20.2552	0.00599655			
	10.	4024	ppymilk=12md_s	2.25104e.09	2.25104e.08	0.00208622	250.959	brain dawa wa	16222.0	210.000	0.0322525		Visualization	
	11.	6451	niconnic-100a_	2.231040-00	2.231040-08	2597.05	2230.030	brain up up	16322.0	200,164	0.0233333		Cluster Based on Significant miRNAs	
	12.	7579	vtr.miD-124 et	2.474496-09	2.273070-00	3471.03	3471.03	brain up vo	10233.3	203.104	0.0533102		✓ miRNA Integration	
	14	4074	mdo.miP-1 et	2.57466.09	2.551460.09	0.00105015	.052.245	brain down yr	15221.6	146 977	0.0393102		Combine miRNAs with mRNA Targets	
	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		Find Overrepresented miRNA Target Sets	
	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		Correlate miRNA and mRNA Data	
	17.	3950	lla-miR-133a st	3.31359e-08	3.31359e-08	0.00286725	-348.767	brain down vs	13453	107.005	0.0318161		Biological Interpretation	
	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		Cone Set Analysis	
	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		Pathway Analysis	
	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			
	75	5747	moa.miD74 et	6 57071a.08	6 57071a.08	0 305780	-3 22023	brain down ve	0557 57	4 38303	0.00183534			
< >	Rows:	7815 Columns:	11 <									> ~		
3													L.	

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

🌮 Lie	st Manager				_		×
List	Spreadsheet Name	List Info Venn Diagram	ANOVA Streamlined	Advanced			
A	1 (Affy_miR_BrainHeart_ir	Single factor: find genes t	that vary across all samp	les upon single factor			
В	1/ANOVA-1way (ANOVA	Name		Setting		# Pass	
с	2 (Affy_HuGeneST_BrainH	O Tissue					
D	2/1 (ANOVAResults gene)						
		Contrast: find genes that	change between two ca	tegories			
		Name		Setting		# Pass	
		Iteration brain vs. heart		Have Any Change	~	1404	
		Configuration for "brain v	s. heart"		# Pa	ss: 1404	
		Include size of the d	hange Chi	ange > 2 OR Change < -2			
		Include significance	of the change p-\	value with FDR v < 0.05			
		1404 genes passed the sp You are about to create a Have Any Chan	pecified criteria. list of genes that ge in brain relative to he	art with fdr (step up) < 0.05, Change >	2 or Change <	-2	*
<	>	Save list as: brain vs	s. heart Br	rowse Create	[Configu	re
						Close	e

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).

Partek Genomics Suite - 1/brain_vs_heart (brain_vs_heart)	rain vs.	heart)											- 1	×
<u>File Edit Transform View Stat</u> Filter T	ools	Window Cu	stom <u>H</u> elp										Workflows miDNA Expransion	
Analysis M. Conttan Dist M.													miRNA Expression	×
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			•••	-									Import Samples	
1 (Affy_miR_BrainHeart_intensities) ^ _	Curren	t Selection 268	9				6	-		-		<u>^</u>	Add Sample Attributes	
ANOVA-1way (ANOVAResults)		1. Column #	2. Probeset ID	3. p-value(Tissue)	4. p-value(brain	5. Ratio(brain vs.	6. Fold-Change(br	7. Fold-Change(br	8. F(Tissue)	9. SS(Tissue)	SS(Error)	1	View Sample Information	
Drain_vs_heart (brain vs. heart)					vs. heart)	heart)	ain vs. heart)	ain vs. heart) (Description)				-	V QA/QC	
1 (ANOVAResults gene)	1.	2689	fru-miR-128 st	1.85766e-10	1.85766e-10	21.6101	21.6101	brain up vs	179715	29.4857	0.000656276	1	PCA Scatter Plot	
	2.	5453	odi-miR-1c_st	9.41986e-10	9.41986e-10	0.000968415	-1032.62	brain down vs	79806	150.363	0.00753642	i	Correla Day & Whiches Obst	· ·
	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	1	Sample Box & whiskers Chart	
	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	1	Sample Histogram	
	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	i	✓ Analysis	
	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	1	Detect Differentially Expressed miRN	As 🗸 🗸
	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	1	View Sources of Variation	
	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	1	Create List	
	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	1		
	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	1	Cluster Based on Significant miPNAs	
	11.	4034	mdo-miR-133a_	2.25104e-08	2.25104e-08	0.00398632	-250.858	brain down vs	16322.8	95.2987	0.0233535	1	Cluster Dased on Significant michael	
	12.	6451	ptr-miR-124a_s	2.27587e-08	2.27587e-08	3587.05	3587.05	brain up vs	16233.5	209.164	0.0515387	1	miRNA Integration	
	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	1	Combine miRNAs with mRNA Target	3
	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	1	Find Overrepresented miRNA Target	Sets
	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	1	Correlate miRNA and mRNA Data	
	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	1	✓ Biological Interpretation	
	17.	3950	lla-miR-133a_st	3.31359e-08	3.31359e-08	0.00286725	-348.767	brain down vs	13453	107.005	0.0318161	1	Gene Set Analysis	
	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	1	Bathway Analysis	
	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	1	Fattiway Analysis	
	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	1		
	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	1		
	22.	2506	dre-miR-199-sta	5.75155e-08	5.75155e-08	0.0508008	-19.684/	brain down vs	10210.4	27.7222	0.0108604	1		
	23.	2028	ure-mik-9_st	5.985186-08	5.985186-08	134./31	214 200	brain up vs	10009	/5.061	0.0299973			
	24.	5242	mpo.miR-24_=t	6 570710-00	6 570710-00	0.00318158	-2 22022	brain down vs	9030.54	4 29202	0.00183534			
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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.

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(Affy_HuGeneST_BrainHeart_GeneInt	_							(Description)					✓ QA/QC	
1 (ANOVAResults gene)	1.	6105	ppy-miR-124a_st	1.72471e-08	1.72471e-08	4101.24	4101.24	brain up vs	18648.3	216.066	0.0463454		PCA Scatter Plot	
	2.	3139	hsa-miR-124_st	1.43893e-09	1.43893e-09	4087.94	4087.94	brain up vs	64570.4	215.898	0.0133744		Sample Box & Whiskers Chart	
	3.	3947	lla-miR-124a_st	1.00365e-07	1.00365e-07	4035.1	4035.1	brain up vs	7728.55	215.223	0.111391		Sample Histogram	
	4.	7217	ssc-miR-124a_st	1.00179e-07	1.00179e-07	3773.62	3773.62	brain up vs	7735.72	211.763	0.109499			
	5.	6580	rno-miR-124_st	1.07396e-07	1.07396e-07	3635.95	3635.95	brain up vs	7471.14	209.856	0.112356		Detect Differentially Europeand miDN/	
	6.	6451	ptr-miR-124a_st	2.27587e-08	2.27587e-08	3587.05	3587.05	brain up vs	16233.5	209.164	0.0515387		Detect Differentially Expressed miRIV	٨S
	7.	4228	mml-miR-124a_st	5.38356e-07	5.38356e-07	3535.24	3535.24	brain up vs	3335.09	208.421	0.249974		View Sources of Variation	
	8.	7042	sme-miR-124c_st	1.91116e-07	1.91116e-07	3502.53	3502.53	brain up vs	5599.75	207.947	0.14854		Create List	
	9.	1621	cbr-miR-124_st	6.51605e-07	6.51605e-07	3490.76	3490.76	brain up vs	3031.14	207.776	0.274188		Visualization	
	10.	7578	xtr-miR-124_st	2.47448e-08	2.47448e-08	3471.03	3471.03	brain up vs	15568.3	207.487	0.0533102		Cluster Based on Significant miRNAs	
	11.	7323	tni-miR-124_st	2.16154e-07	2.16154e-07	3390.97	3390.97	brain up vs	5265.25	206.301	0.156726		miPNA Integration	
	12.	2432	dre-miR-124_st	1.46988e-06	1.46988e-06	3384.91	3384.91	brain up vs	2017.05	206.21	0.408934			
	13.	1509	bta-miR-124a_st	3.64338e-08	3.64338e-08	3364.43	3364.43	brain up vs	12829.5	205.902	0.0641963		Combine miRNAs with mRNA Targets	
	14.	1122	age-miR-124a_st	1.18828e-06	1.18828e-06	3350.42	3350.42	brain up vs	2243.74	205.691	0.366693		Find Overrepresented miRNA Target S	iets
	15.	4028	mdo-miR-124a_st	1.10697e-06	1.10697e-06	3270.29	3270.29	brain up vs	2324.8	204.465	0.351799		Correlate miRNA and mRNA Data	
	16.	1174	ame-miR-124_st	7.7427e-08	7.7427e-08	3259.92	3259.92	brain up vs	8799.64	204.305	0.0928698		✓ Biological Interpretation	
	17.	5753	ppa-miR-124a_st	2.43602e-06	2.43602e-06	3228.44	3228.44	brain up vs	1566.07	203.815	0.520577		Gene Set Analysis	
	18.	7040	sme-miR-124b_st	1.3826e-07	1.3826e-07	3153.39	3153.39	brain up vs	6584.27	202.63	0.1231			
	19.	4730	mmu-miR-124_st	6.23674e-06	6.23674e-06	3121	3121	brain up vs	977.505	202.111	0.82705		Pathway Analysis	
	20.	2347	dps-miR-124_st	1.19896e-07	1.19896e-07	3091.91	3091.91	brain up vs	7070.81	201.641	0.11407			
	21.	2167	csa-miR-124_st	1.31008e-06	1.31008e-06	3016.08	3016.08	brain up vs	2136.73	200.397	0.375147			
	22.	1881	cfa-miR-124_st	8.07557e-06	8.07557e-06	2994.77	2994.77	brain up vs	858.631	200.042	0.931913			
	23.	2919	ggo-miR-124a_st	1.4053e-07	1.4053e-07	2824.15	2824.15	brain up vs	6530.84	197.121	0.120732			
	24.	1078	aga-miR-124_st	4.59817e-08	4.59817e-08	2765.3	2765.3	brain up vs	11419.7	196.078	0.0686803			
~	75	70.30	ema_miD_174a_et	1 330120-06	1 320120-06	2747 79	2747 79	brain un ve	2128 57	105 763	0 367877			
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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

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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

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

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Find What:	Homo sapiens	~
Replace With:		
Search:	By Columns 🗸 🔿 All columns	
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Find	d Next Select All	Replace All Close

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.

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1 (ANOVAResults gene)	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	PCA Scatter Plot		
	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	Sample Box & Wh	iskers Chart	
	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	Cample Histogram		
	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	Sample Histogram		
	5.	3862	hsa-miR-885-5p_s	Homo sapiens	4.22467e-05	4.22467e-05	185.963	185.963	brain up vs	373.528	85.252	✓ Analysis		
	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	Detect Differential	y Expressed miRN	iAs 🗸
	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	View Sources of V	ariation	
	8.	3584	hsa-miR-499-5p_s	Homo sapiens	2.26823e-06	2.26823e-06	0.00829795	-120.512	brain down vs	1623.08	71.685	Create List		
	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	Visualization		
	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	Cluster Based on	Significant miRNA	\$
	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	✓ miPNA Intograti	on	
	12.	3192	hsa-miR-129-3p_s	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	Combine miRNAs	with mRNA Target	s
	14.	3522	hsa-miR-383_st	Homo sapiens	0.000261715	0.000261715	57.0328	57.0328	brain up vs	148.085	51.0485	Find Overrepresen	ied miRNA Target	Sets
	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	Correlate miRNA a	ind mRNA Data	
	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	✓ Biological Inter	pretation	
	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	Gene Set Analysis	3	
	18.	3552	hsa-miR-452_st	Homo sapiens	0.000109739	0.000109739	0.0187499	-53.3335	brain down vs	230.498	49.3692	Dathway Analysis		
	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	T attiway Analysis		
	20.	3543	hsa-miR-433_st	Homo sapiens	1.91458e-05	1.91458e-05	46.2205	46.2205	brain up vs	556.4/6	45.879			
	21.	3193	nsa-miR-129-5p_s	Homo sapiens	9.953/68-05	9.953/68-05	45.5802	45.5802	brain up vs	242.187	45.5457			
	22.	3449	hsa-miR-323-3p_s	Homo sapiens	0.000199975	0.000199975	44.5337	44.5337	brain up vs	169.887	44.9934			
	23.	869	HBI1-52-33_X_st	Homo sapiens	9.95358-06	9.95358-06	44.0713	44.0713	brain up vs	//3.0/2	44.7463			
	24.	3570	nsa-miR-490-3p_s	Homo sapiens	6.26952e-05	6.26952e-05	0.0251/46	-39.7226	brain down vs	306.025	42.3242			
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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).

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brain vs heart human (brain vs. h				Scientific Name		vs. neart)	neart)	ain vs. neart)	(Description)				∨ QA/QC	
2 (Affy_HuGeneST_BrainHeart_GeneInt	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		PCA Scatter Plot	
1 (ANOVAResults gene)	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		Sample Box & Whiskers Chart	
	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		Sample Histogram	
	5.	3862	hsa-miR-885-5p_s	Homo sapiens	4.22467e-05	4.22467e-05	185.963	185.963	brain up vs	373.528	85.252		✓ Analysis	
	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		Detect Differentially Expressed miRNAs	
	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		View Sources of Variation	
	8.	3584	hsa-miR-499-5p_s	Homo sapiens	2.26823e-06	2.26823e-06	0.00829795	-120.512	brain down vs	1623.08	71.685		Create List	
	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		Visualization	
	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		Clueter Based on Significant miPNAs	
	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_s	Homo sapiens	3.64626e-05	3.64626e-05	68.0845	68.0845	brain up vs	402.319	55.6185		MIRNA Integration	
	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		Combine miRNAs with mRNA Targets	
	14.	3522	hsa-miR-383_st	Homo sapiens	0.000261715	0.000261715	57.0328	57.0328	brain up vs	148.085	51.0485		Find Overrepresented miRNA Target Sets	
	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		Correlate miRNA and mRNA Data	
	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		✓ Biological Interpretation	
	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		Gene Set Analysis	
		3552	hsa-miR-452_st	Homo sapiens	0.000109739	0.000109739	0.0187499	-53.3335	brain down vs	230.498	49.3692		Ballana Aastais	
	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		Pathway Analysis	
	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_s	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_s	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_s	Homo sapiens	6.26952e-05	6.26952e-05	0.0251746	-39.7226	brain down vs	306.025	42.3242			
· · · · · · · · · · · · · · · · · · ·		251 Columne: 1	HRIT_57_7 v et	Homo canienc	7 127220.05	7 122220.05	30 5521	30 5571	brain un ve	786 711	47 7754			
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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