Integrate miRNA and Gene Expression data

- Finding putative genes regulated by miRNAs
- · Finding overrepresented miRNA targets sets from gene expression data
- Combine miRNAs with mRNA target genes
- Correlating 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)

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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)

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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).

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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

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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

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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 trageted 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.

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	16.	bta-mir-182	3.32797e-08	52	42	10	1127	349	18995		✓ Biological Interpretation	
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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)

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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

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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).

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	11.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Cluster Based on Significa	Int mirinas	
	12.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		✓ miRNA Integration		
	13.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Combine miRNAs with mF	(NA Targets	✓
	14.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Find Overrepresented miR	NA Target Sets	
	15.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Correlate miRNA and mRI	A Data	
	16.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		✓ Biological Interpretation	n	
	17.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Gene Set Analysis		
	18.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Built and a lai		
	19.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983		Pathway Analysis		
	20.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983				
	21.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983				
	22.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983				
	23.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983				
	24.	3225	hsa-miR-133b_s	s Homo sapiens	7.65502e-06	7.65502e-06	0.0051452	-194.356	brain down vs	881.992	86.6983				
×	Rowe	2006 2: 15549 Columner	33 x	Home coniene	7 655000 06	7 655070 06	0.0051450	104 952	besin down we	001 000	02 2000				
< >	J	a 100 io Columns.										> v			
\odot															

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

🧬 Correlate microRNA-mR	NA data	×
This dialog correlates microRN/ data of gene targets using Pea	A expression data with e arson's and Spearman's (expression correlation.
Target Database		
Database Name:	TargetScan7.1	~ ?
microRNA Spreadsheet		
Spreadsheet Name:	1 (Affy_miR_BrainHea	rt_i v 🕜
mRNA Spreadsheet		
Spreadsheet Name:	2 (Affy_HuGeneST_B	rain' 🗸 ?
Result file		
correlation.txt		Browse
	ок	Cancel

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)

🤣 Choose Sample ID Columns		×										
The sample ID column is required for integrat IDs must match the sample IDs from the spre	The sample ID column is required for integrated analysis (using the filename is not recommended). The specified sample IDs must match the sample IDs from the spreadsheet with which you want to integrate. Sample IDs are case sensitive.											
Spreadsheet	Sample ID Column	First Sample ID										
1 (Affy_miR_BrainHeart_intensities)	6. SampleID v	B1										
2 (Affy_HuGeneST_BrainHeart_GeneInter	6. SampleID v	B1										
		OK Cancel										

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 its target gene.

😰 Partek Genomics Suite - correlation (correla	ition.txt)											- 0	\times
												Workflows miRNA Expression	
Analysis × Scatter Plot ×												miRNA Expression	\rightarrow
🖻 🚘 🔲 🖺 🗍 🛵 🖬 🖡	<u></u>		– 0									✓ Import	
				·								Import Samples	
□ 1 (Affy_miR_BrainHeart_intensities) ^	Current Select	ion xtr-miR-148a_	st								^	Add Sample Attributes	
ANOVA-1way (ANOVAResults)		1.	2.	3. Gene Symbol	4. MicroPNA Name	5. Dearcon	6.	7.	8. p-value/Spearm			View Cample Information	
brain_vs_heart (brain vs. heart)		(Affy_miR_Brain	2 (Affy_HuGeneS	Gene Symbol	MICLOKINA INditie	correlation	correlation)	rank correlation	an's rank			View Sample Information	
brain_vs_heart_human (brain vs. he		Heart_intensitie	T_BrainHeart_G			coefficient		coefficient	correlation)			✓ QA/QC	
2 (Affy_HuGeneST_BrainHeart_GeneInt	1.	xtr-miR-148a_st	8163882	RAB14	xtr-mir-148a	-0.999932	6.8853e-09	-1	0			PCA Scatter Plot	•
1 (ANOVAResults gene)	2.	xtr-miR-145_st	7972650	FGF14	xtr-mir-145	-0.999878	2.24222e-08	-1	0			Sample Box & Whiskers Chart	
Brain_vs_Heart (brain vs. heart gen	3.	mml-miR-22_st	8011774	CAMTA2	mml-mir-22	-0.999877	2.26369e-08	-1	0			Sample Histogram	
3 (PutativeGenes)	4.	bta-miR-145_st	8152376	CSMD3	bta-mir-145	-0.999865	2.72972e-08	-0.942857	0.00480466				
5 (combine txt)	5.	cfa-miR-22_st	8011774	CAMTA2	cfa-mir-22	-0.999854	3.20989e-08	-0.942857	0.00480466				
correlation (correlation bt)	6.	ptr-miR-107_st	7903092	FNBP 1L	ptr-mir-107	-0.99975	9.38802e-08	-1	0			Detect Differentially Expressed miRNAs	•
conclution (conclutionitaty)	7.	ptr-miR-145_st	7925457	RGS7	ptr-mir-145	-0.999729	1.10131e-07	-0.885714	0.0188455			View Sources of Variation	
	8.	ptr-miR-154_st	8084742	LPP	ptr-mir-154	-0.999643	1.90949e-07	-1	0			Create List	
	9.	bta-miR-19b_st	7951873	SIK3	bta-mir-19b	-0.999554	2.98466e-07	-0.942857	0.00480466			Visualization	
	10.	ptr-miR-24_st	8097449	PCDH10	ptr-mir-24	-0.999554	2.98293e-07	-0.885714	0.0188455			Cluster Record on Significant miDNAs	
	11.	cfa-miR-143_st	8078920	MOBP	cfa-mir-143	-0.999548	3.05993e-07	-0.828571	0.0415627			Cluster Based on Significant mikivas	
	12.	bta-miR-10b_st	7979529	KCNH5	bta-mir-10b	-0.999545	3.10703e-07	-0.942857	0.00480466			✓ miRNA Integration	
	13.	xtr-miR-130c_st	8019074	NPTX1	xtr-mir-130c	-0.999543	3.13407e-07	-0.942857	0.00480466			Combine miRNAs with mRNA Targets	•
	14.	ptr-miR-143_st	7971526	HTR2A	ptr-mir-143	-0.99953	3.31013e-07	-0.771429	0.0723965			Find Overrepresented miRNA Target Sets	s 💊
	15.	mml-miR-181d_s	8070297	ERG	mml-mir-181d	-0.999525	3.38954e-07	-1	0			Correlate miRNA and mRNA Data	
	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			Gene Set Analysis	
	19.	bta-miR-124a_s	8098263	PALLD	bta-mir-124a	-0.999437	4.74597e-07	-0.771429	0.0723965			Pathway Analysis	
	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				
~	ne	hts miD 325 at	NUNCONO	DACAD	hts mir 225	0.00020	6 03009- 07	0.049057	0.00490466				
< >	Rows: 261966	Columns: 8 <								>	~		

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

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

