Tutorial

QIAseq miRNA Quantification

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Sample to Insight

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QIAseq miRNA Quantification

This tutorial uses the capabilities of CLC Genomics Workbench and the Biomedical Genomics Analysis plugin to quantify miRNA in tumor tissues. We chose to work with a reduced data set from the Decmann et al., 2018 publication, using 6 out of the 30 formalin-fixed, paraffin-embedded (FFPE) archived tissue samples, 3 of adrenal myelolipoma (AML) and 3 of adrenocortical carcinoma (ACC). Adrenal Myelolipoma is a benign primary adrenal neoplasm that is difficult to distinguish from Adrenocortical Carcinoma which has very poor prognosis. The study investigates miRNA expression in AML versus ACC in the hope to find biomarkers that would facilitate diagnosis.

This tutorial covers in just a few steps all the following:

- Import Illumina reads and a metadata table in the Workbench.
- Associate the metadata with the reads.
- Quantify miRNA expression with the **QIAseq miRNA Quantification** ready-to-use workflow.
- Visualize results using the **Create Combined Report** tool and the **QIAseq miRNA Differential Expression** workflow.

This tutorial with the reduced data set can be run within 20 min on a regular laptop. Note that the full data set of the study can always be downloaded from the tool **Search for Reads in SRA**.

Prerequisites For this tutorial, you must be working with the CLC Genomics Workbench with Biomedical Genomics Analysis plugin installed. How to install plugins is described here: `http://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Install.html`.

Download and import data Go through the following steps to download and import the data into the Workbench.

1. Download the sample data from our website: `http://resources.qiagenbioinformatics.com/testdata/miRNA_tutorial.zip` and save it on your computer.
2. Start the workbench.
3. Import the reads and the metadata table via the toolbar: **Import** () | **Standard Import**.
4. Select the `miRNA_tutorial.zip` file and leave the option to "Automatic import". Click **Next**.
5. Select the location in the Navigation Area of your Workbench where the data should be saved, and click **Finish**.
6. Before starting the analysis, the metadata table and the reads need to be associated. Open the `miRNA_AML_tutorial` table and click on the button **Associate Data...** highlighted in red in figure 1. Choose the option "Associate Data Automatically..." in the drop-down menu.
7. In the next wizard that opens, choose the read you just imported (figure 2) and click **Next**.

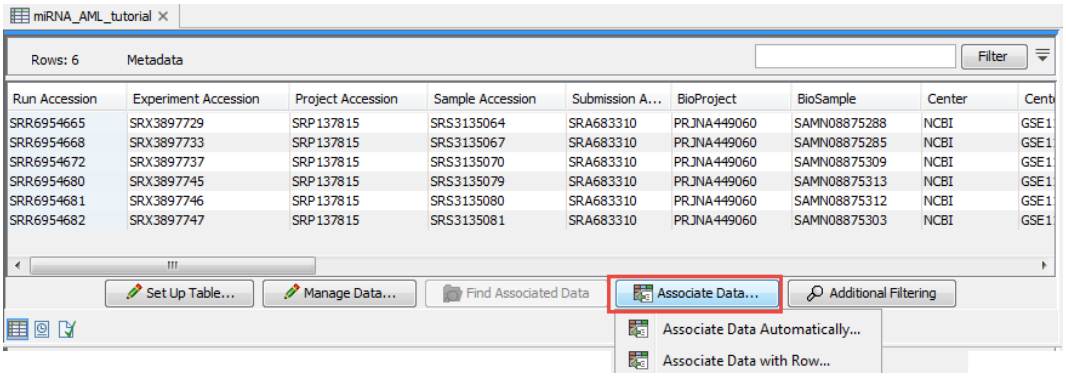

Figure 1: *Associate metadata and reads before running the analyses.*

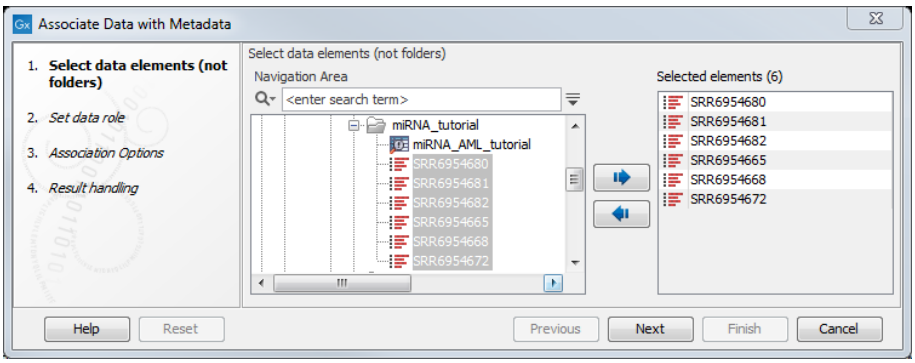

Figure 2: *Select the reads for association.*

8. In the next dialogs, leave the data role to **Sample data** and the matching scheme to **Exact**. You can then click **Finish**. Your reads and the table are now associated, association that is required for the Differential Expression analysis that is run at the end of the tutorial.

Run QIAseq miRNA Quantification

The QIAseq miRNA Quantification ready-to-use workflow can be found in the Toolbox here:

Ready-to-Use Workflows | **Small RNA sequencing** | **QIAseq miRNA Quantification**

1. Double-click on the QIAseq miRNA Quantification ready-to-use workflow to run the analysis.

   If you are connected to a CLC Server via your Workbench, you will be asked where you would like to run the analysis.

2. Select the sequencing reads that should be analyzed (figure 3). Remember to check the batch option - as highlighted in red in the figure - before clicking **Next**.

3. Select the QIAseq Small RNA Reference Data Set from the Reference manager (figure 4). You can download the data set to the CLC_References folder of the Navigation Area now if you had not done so before.

4. Leave all parameters for the **Create UMI Reads for miRNA** tool as they are set by default (figure 5) and just click **Next**. These parameters have been configured to work well with reads that were sequenced with the Illumina technology. However, note that when working

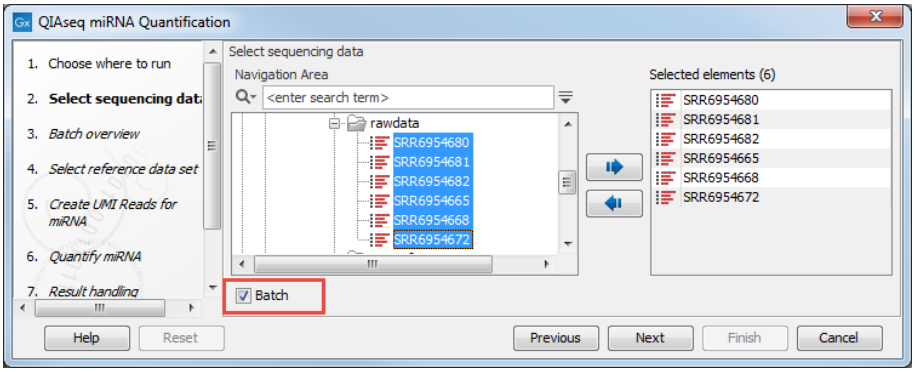

Figure 3: *Select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.*

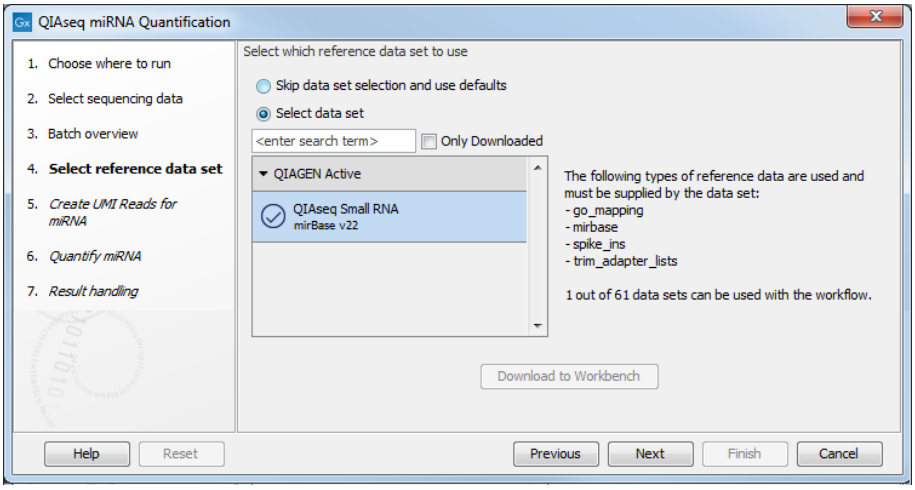

Figure 4: *Select the QIAseq Small RNA reference data set.*

with Ion Torrent reads, we recommend to enable all options and change the "Maximum differences in small RNA sequences" to 2.

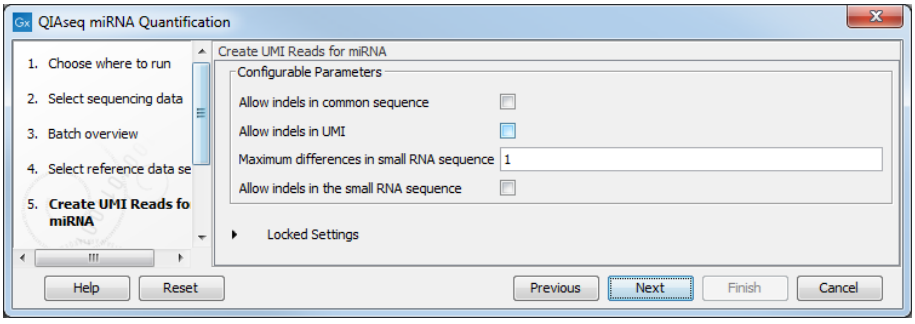

Figure 5: *Parameters for Create UMI Reads for miRNA.*

5. The **Quantify miRNA** dialog is also pre-configured adequately for the current data (figure 6). The data we are using is from human samples so only Homo sapiens need to be selected in the Prioritized species list. Spike-ins are not included in the data but the option to enable them can be left checked. Click **Next**.

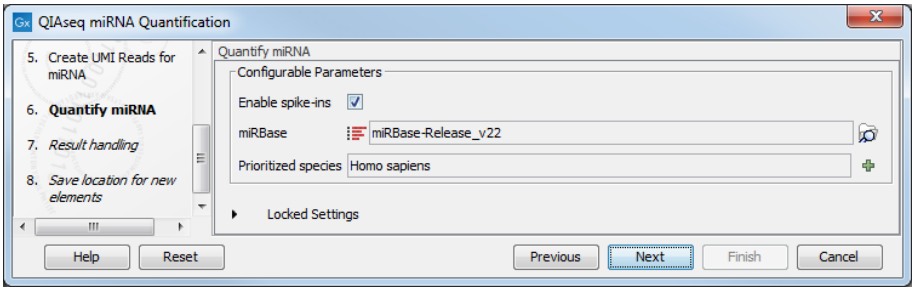

Figure 6: *Parameters for the Quantify miRNA tool.*

6. Finally, in the last wizard step, choose to **Save** the results of the workflow in one subfolder per batch unit, and specify the location of your choice in the Navigation Area before clicking **Finish**.

Output from the QIAseq miRNA Quantification workflow

The QIAseq miRNA Quantification workflow produces a folder containing a series of reports, and two expression tables for each batch unit (figure 7).

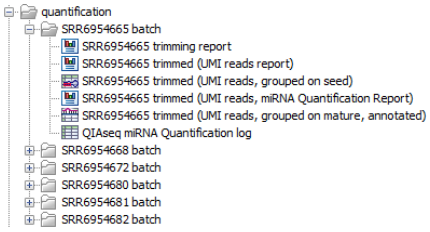

Figure 7: *Outputs from the QIAseq miRNA Quantification workflow.*

In the **Grouped on mature** expression table (), there is a row for each mature miRNA in the database, including those for which the expression is zero. Double click on a row to open a unique reads alignment. The alignment shows all possible unique reads that have aligned to a specific miRNA from the database. Mismatches to the mature reference are highlighted in the alignment and recapitulated in their name.

The **Grouped on seed** expression table () has a row for each seed sequenced. This file is useful for further analysis in Ingenuity Pathway Analysis (IPA) where it can be uploaded if the Ingenuity Pathway Analysis plugin is installed and you have an active license for IPA (this however is outside the scope of this tutorial).

Finally, each batch unit will output three reports, including the **Quantification report**. Although it is possible to review each report separately, we can also use the Create Combined miRNA Report tool to compile all of them into one. The tool can be found here:

Tools | **RNA-Seq Analysis** | **miRNA Analysis** | **Create Combined miRNA Report**

First, select the reports that should be combined (figure 8). We chose here to combine all reports from all batch units to get an overview of UMI statistics, mapping and annotation summary and miRNA quantification all in one report.

In the next dialog we choose to keep the name of our samples as they are and not to substitute

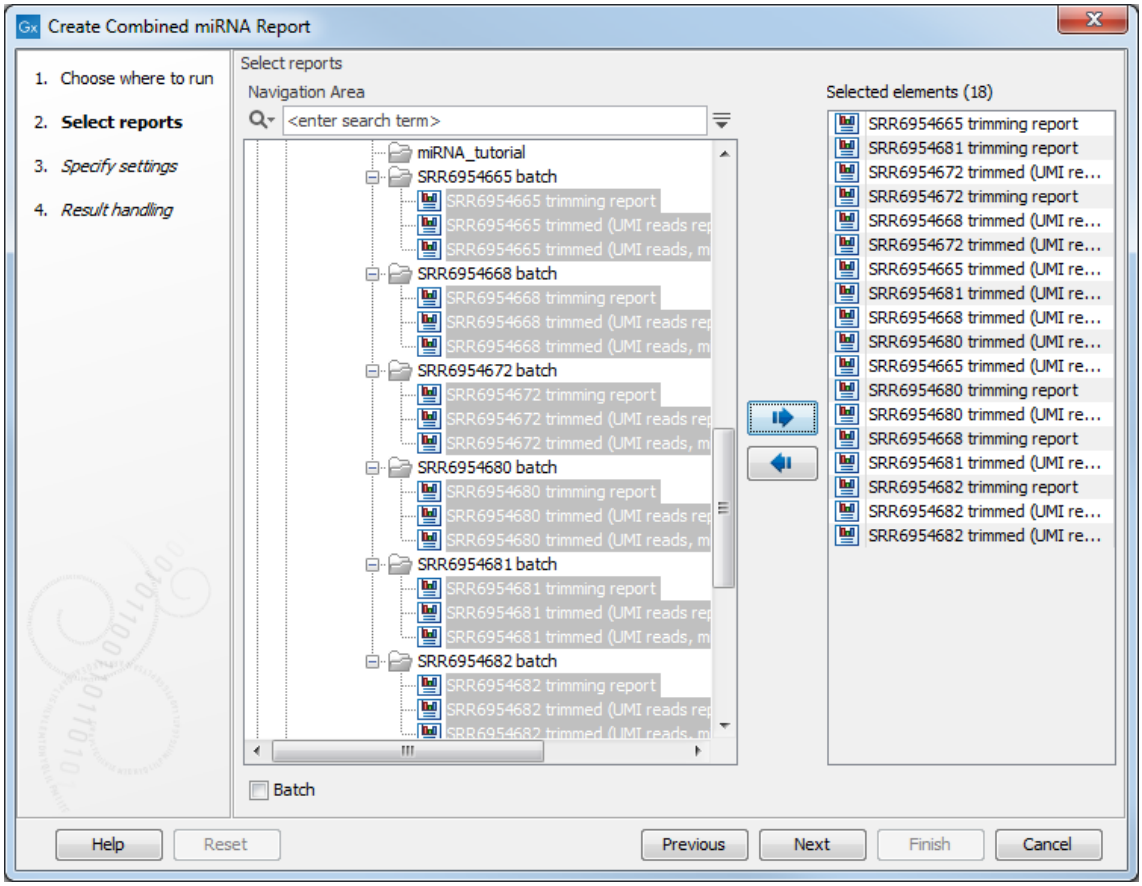

Figure 8: *Combined miRNA trimming, UMI reads and quantification reports into one.*

them for a shorter alias. We then choose to **Open** the report before clicking **Finish**.

Several tables summarize the data and compile the top 20 mature sequences, the top 20 seeds and top 20 novel seeds with counts for the different samples. A question mark ? in these tables indicates when a feature is not among the top 20 novel seeds from a particular sample.

Run QIAseq miRNA Differential Expression

To continue our investigation, we will use the **QIAseq miRNA Differential Expression** workflow.

The QIAseq miRNA Differential Expression ready-to-use workflow can be found in the Toolbox here:

> **Ready-to-Use Workflows | Small RNA sequencing | QIAseq miRNA Differential Expression**

1. Double-click on the QIAseq miRNA Differential Expression ready-to-use workflow to run the analysis.

   If you are connected to a CLC Server via your Workbench, you will be asked where you would like to run the analysis.

2. Select the expression tables that should be compared (figure 9) before clicking **Next**. You

can choose either the **mature** (as we do here) or the **seed** expression tables, but not a mix of both.

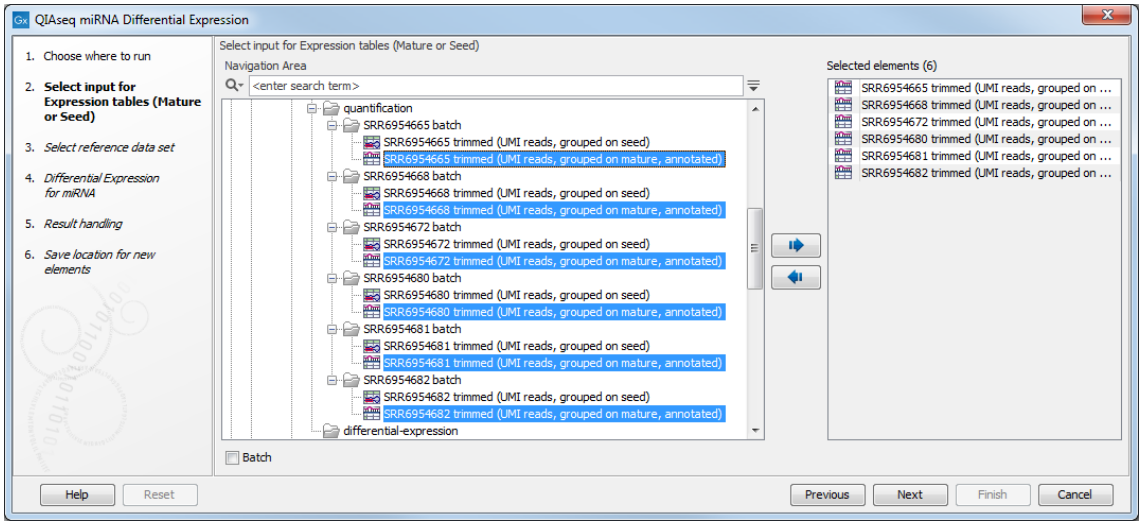

Figure 9: *Select the mature or seed expression tables.*

3. Once again select the QIAseq Small RNA Reference Data Set from the Reference manager (figure 10).

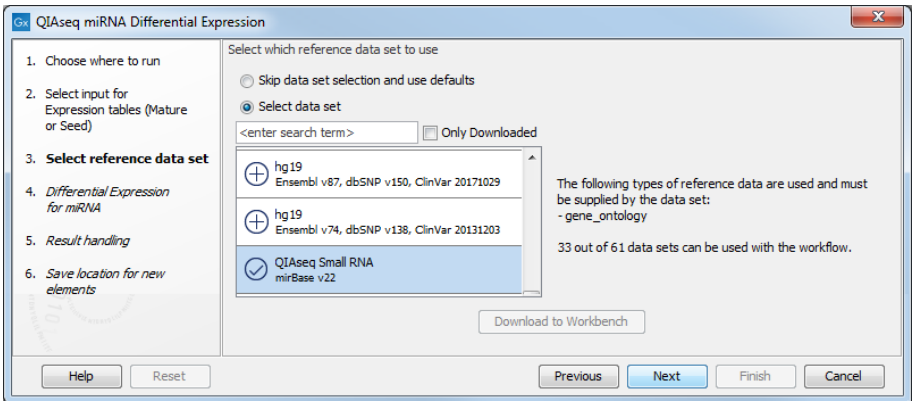

Figure 10: *Select the QIAseq Small RNA reference data set.*

4. In the Differential Expression for miRNA dialog, set the parameters as seen in figure 11.
5. Choose to save the results (shown in figure 12) in the tutorial folder.
6. Open the Heat Map for RNA-seq output (figure 13). The AML and ACC samples are separated, indicating that it may be possible to find miRNA that would be specific to the AML and that could be used for diagnostics.
7. Open the Expression browser (figure 14). In this table, the expressed miRNAs that were chosen in the original paper for validation have been highlighted. In our tutorial data as well do hsa-miR-451a, hsa-miR-486-5p, hsa-miR-363-3p, and hsa-miR-150-5p show significant overexpression in AML relative to ACC.

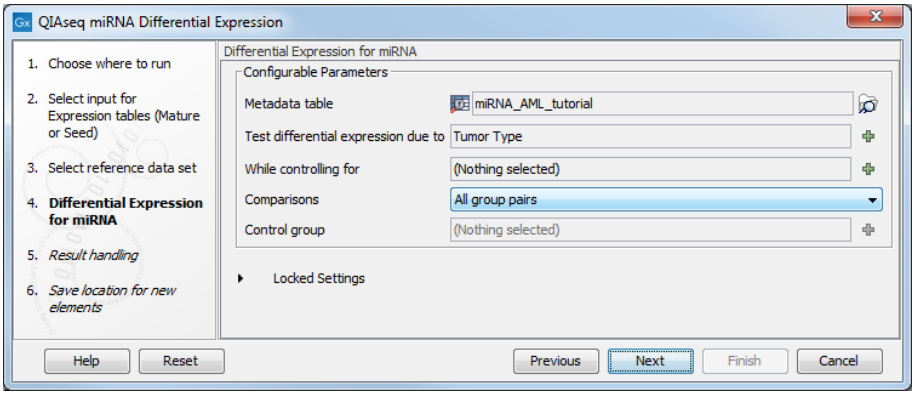

Figure 11: *Parameters for the differential expression analysis.*

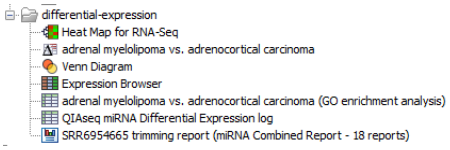

Figure 12: *Outputs of the differential expression analysis.*

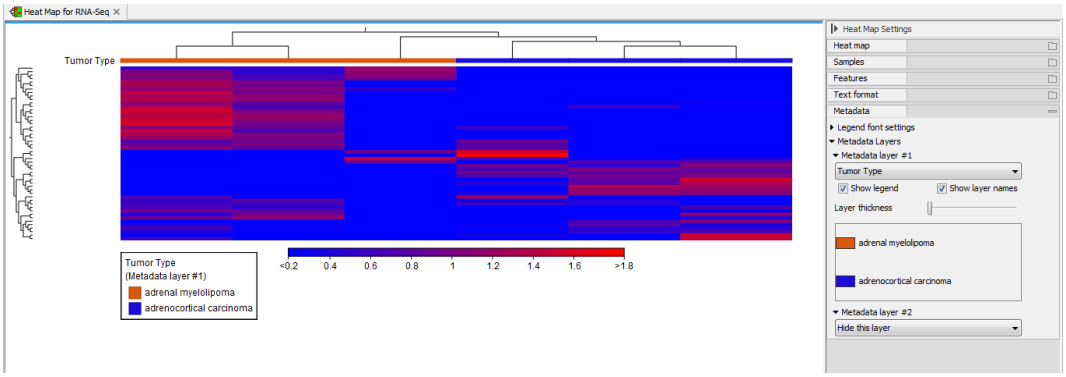

Figure 13: *Heat map for RNA-Seq.*

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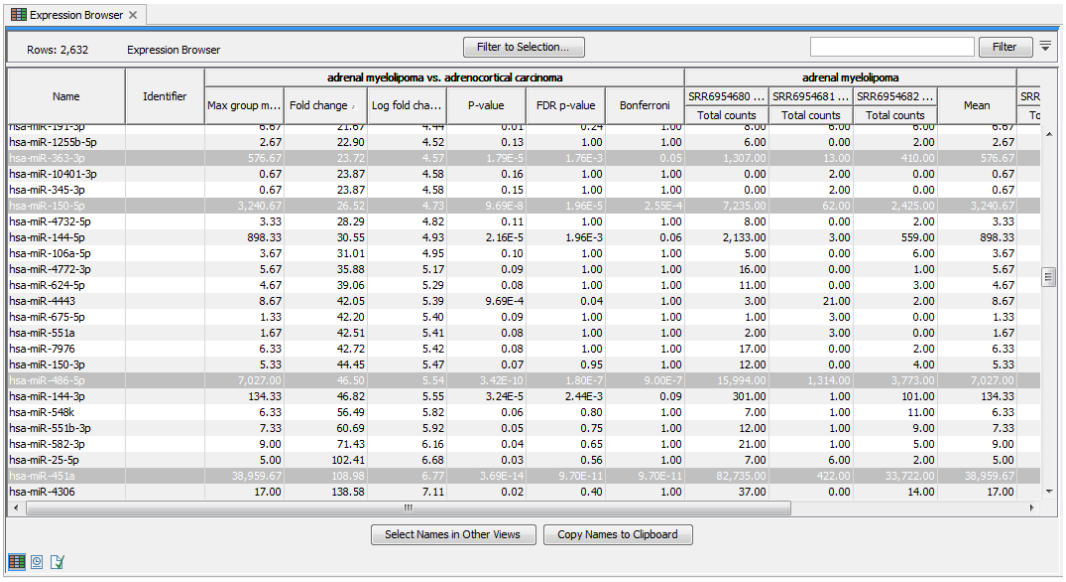

Name	Identifier	adrenal myelolipoma vs. adrenocortical carcinoma						adrenal myelolipoma				
		Max group m...	Fold change	Log fold cha...	P-value	FDR p-value	Bonferroni	SRR6954680 ... Total counts	SRR6954681 ... Total counts	SRR6954682 ... Total counts	Mean	SRR Tc
hsa-miR-191-5p		6.67	21.67	4.44	0.01	0.24	1.00	6.00	6.00	6.00	6.67	
hsa-miR-1255b-5p		2.67	22.90	4.52	0.13	1.00	1.00	6.00	0.00	2.00	2.67	
hsa-miR-363-3p		576.67	23.72	4.57	1.79E-5	1.76E-3	0.05	1,307.00	13.00	410.00	576.67	
hsa-miR-10401-3p		0.67	23.87	4.58	0.16	1.00	1.00	0.00	2.00	0.00	0.67	
hsa-miR-345-3p		0.67	23.87	4.58	0.15	1.00	1.00	0.00	2.00	0.00	0.67	
hsa-miR-150-5p		3,240.67	26.52	4.73	9.69E-8	1.96E-5	2.55E-4	7,235.00	62.00	2,425.00	3,240.67	
hsa-miR-4732-5p		3.33	28.29	4.82	0.11	1.00	1.00	8.00	0.00	2.00	3.33	
hsa-miR-144-5p		898.33	30.55	4.93	2.16E-5	1.96E-3	0.06	2,133.00	3.00	559.00	898.33	
hsa-miR-106a-5p		3.67	31.01	4.95	0.10	1.00	1.00	5.00	0.00	6.00	3.67	
hsa-miR-4772-3p		5.67	35.88	5.17	0.09	1.00	1.00	16.00	0.00	1.00	5.67	
hsa-miR-624-5p		4.67	39.06	5.29	0.08	1.00	1.00	11.00	0.00	3.00	4.67	
hsa-miR-4443		8.67	42.05	5.39	9.69E-4	0.04	1.00	3.00	21.00	2.00	8.67	
hsa-miR-675-5p		1.33	42.20	5.40	0.09	1.00	1.00	1.00	3.00	0.00	1.33	
hsa-miR-551a		1.67	42.51	5.41	0.08	1.00	1.00	2.00	3.00	0.00	1.67	
hsa-miR-7976		6.33	42.72	5.42	0.08	1.00	1.00	17.00	0.00	2.00	6.33	
hsa-miR-150-3p		5.33	44.45	5.47	0.07	0.95	1.00	12.00	0.00	4.00	5.33	
hsa-miR-486-5p		7,027.00	46.50	5.54	3.42E-10	1.80E-7	9.00E-7	15,994.00	1,314.00	3,773.00	7,027.00	
hsa-miR-144-3p		134.33	46.82	5.55	3.24E-5	2.44E-3	0.09	301.00	1.00	101.00	134.33	
hsa-miR-548k		6.33	56.49	5.82	0.06	0.80	1.00	7.00	1.00	11.00	6.33	
hsa-miR-551b-3p		7.33	60.69	5.92	0.05	0.75	1.00	12.00	1.00	9.00	7.33	
hsa-miR-582-3p		9.00	71.43	6.16	0.04	0.65	1.00	21.00	1.00	5.00	9.00	
hsa-miR-25-5p		5.00	102.41	6.68	0.03	0.56	1.00	7.00	6.00	2.00	5.00	
hsa-miR-451a		38,959.67	108.98	6.77	3.69E-14	9.70E-11	9.70E-11	82,735.00	422.00	33,722.00	38,959.67	
hsa-miR-4306		17.00	138.58	7.11	0.02	0.40	1.00	37.00	0.00	14.00	17.00	

Figure 14: *Expression Browser*

Bibliography

[Decmann et al., 2018] Decmann, A., Perge, P., Nyiro, G., Darvasi, O., Likó, I., Borka, K., Micsik, T., Tóth, Z., Bancos, I., Pezzani, R., Iacobone, M., Patócs, A., and Igaz, P. (2018). Microrna expression profiling in adrenal myelolipoma. *Journal of Clinical Endocrinology and Metabolism*, 103(9):3522–3530.