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** template 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**.

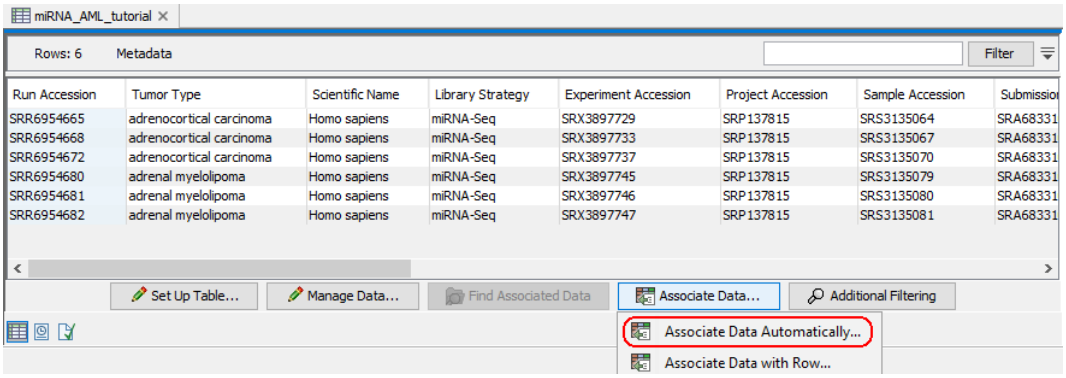


Figure 1: *Associate metadata and reads before running the analyses.*

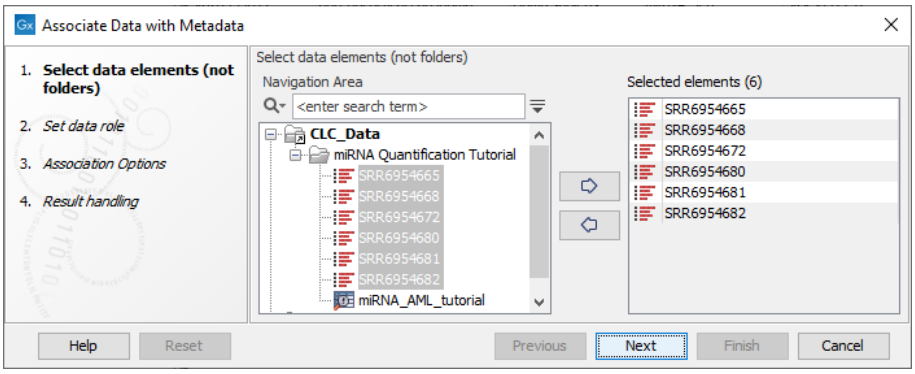


Figure 2: *Select the reads for association.*

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 workflow quantifies the expression in a sample of the miRNAs found in miRBase. The workflow includes a Trim Reads step, but note that this step only affects Ion Torrent reads as Illumina reads do not have the 5' adapter. We are using Illumina reads in this tutorial.

The QIAseq miRNA Quantification template workflow can be found in the Toolbox here:

Template Workflows | **Biomedical Workflows** ( ) | **Small RNA sequencing** | **QIAseq miRNA Quantification**

1. Double-click on the QIAseq miRNA Quantification template 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).

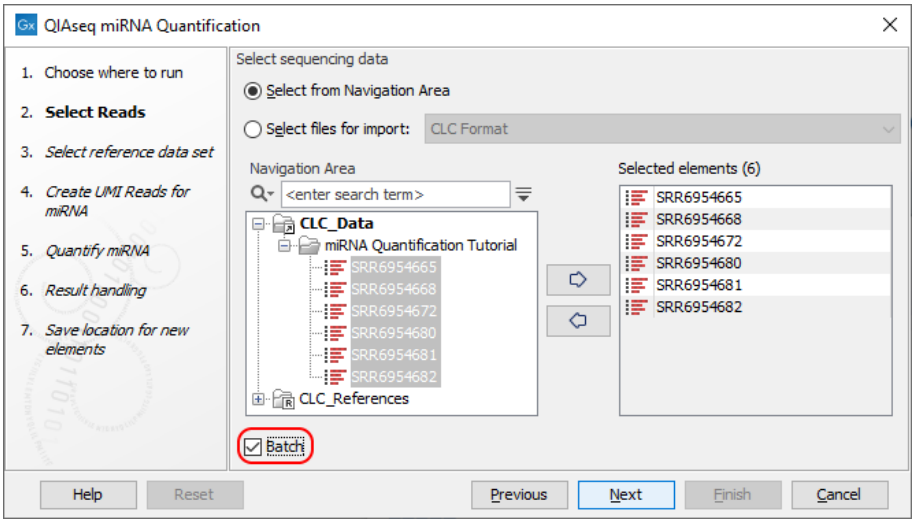


Figure 3: *Select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.*

You can download the data set to the CLC_References folder of the Navigation Area now if you had not done so before.

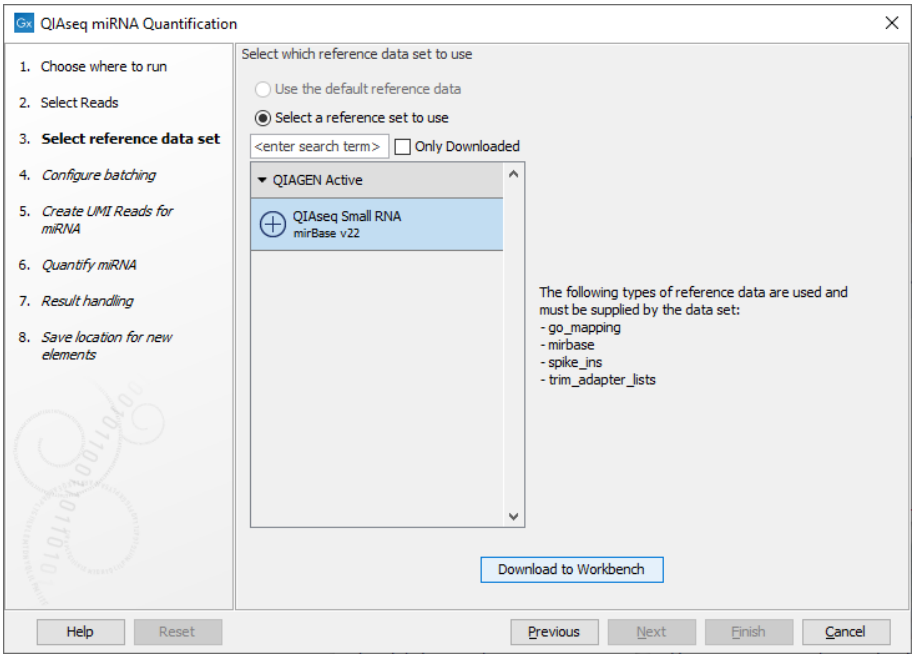


Figure 4: *Select the QIAseq Small RNA reference data set.*

4. Use organization of input data for the **Configure batching** and click **Next**. This mean that the workflow be executed once per sample.
5. The **Batch overview** shows that each batch unit contain one sample as seen in figure 5.
6. Leave all parameters for the **Create UMI Reads for miRNA** tool as they are set by default (figure 6) and just click **Next**. These parameters have been configured to work well with

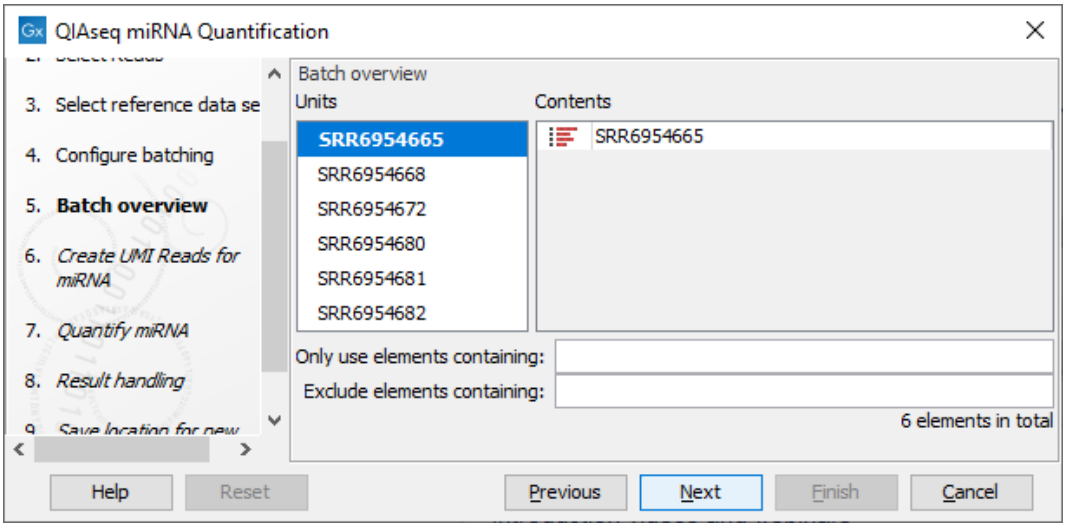


Figure 5: *Overview showing sample content of each batch unit.*

reads that were sequenced with the Illumina technology. However, note that when working with Ion Torrent reads, we recommend to enable all options and change the "Maximum differences in small RNA sequences" to 2.

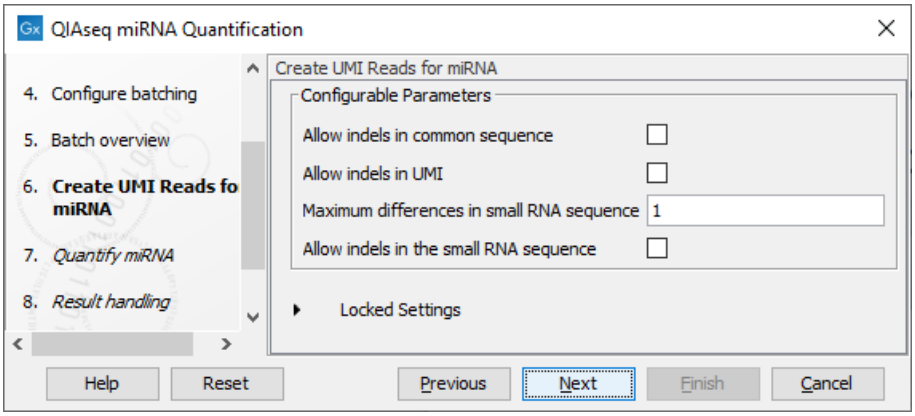


Figure 6: *Parameters for Create UMI Reads for miRNA.*

7. The **Quantify miRNA** dialog is also pre-configured adequately for the current data (figure 7). Spike-ins are not included in the data but the option to enable them can be left checked. The miRBase database are included in the downloaded reference data set in a previous step and therefore pre-configured here. This can be changed if needed but not for this tutorial. The data we are using is from human samples so only Homo sapiens need to be selected in the Prioritized species list. The last parameter provides the option to add sequence lists with additional smallRNA reference databases e.g. piRNAs, tRNAs, rRNAs, mRNAs, lncRNAs. We will leave this empty for now. Click **Next**.

8. 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**.

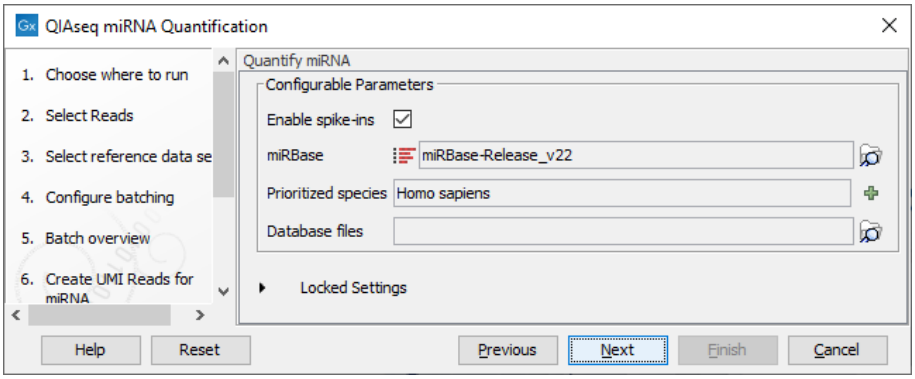


Figure 7: *Parameters for the Quantify miRNA tool.*

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 8).

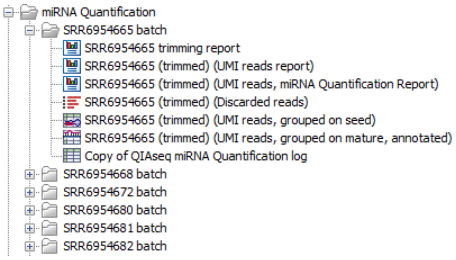


Figure 8: *Outputs from the QIAseq miRNA Quantification workflow.*

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

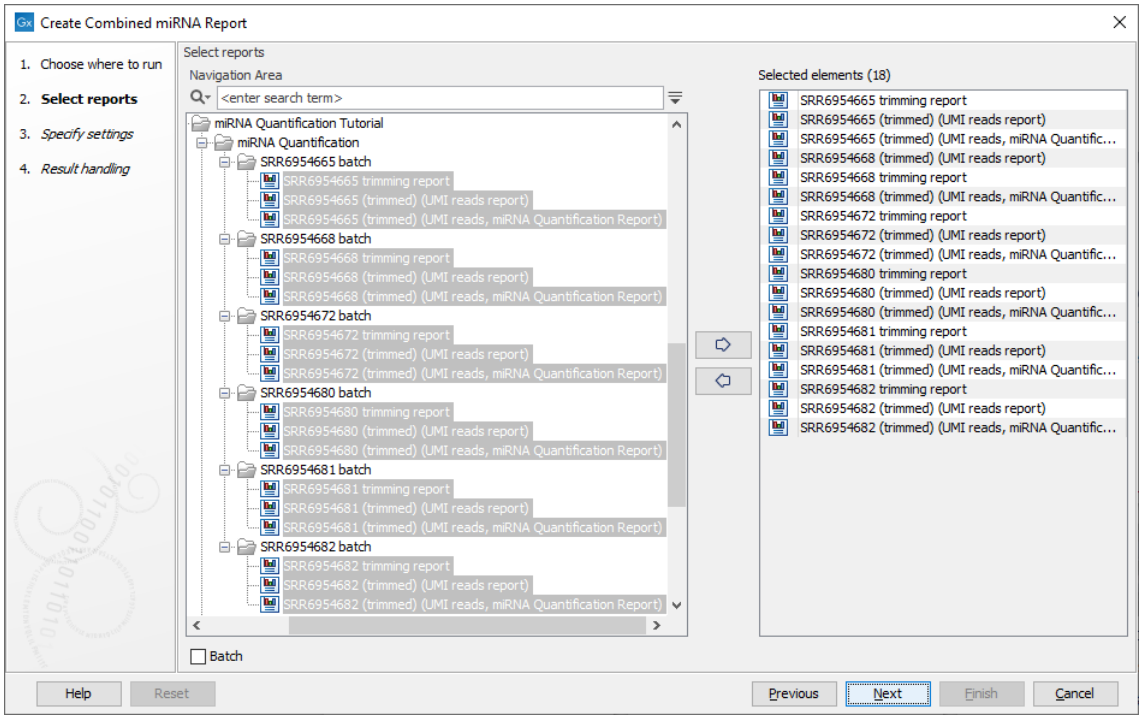


Figure 9: *Combined miRNA quantification reports into one.*

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 template workflow can be found in the Toolbox here:

> **Template Workflows** | **Biomedical Workflows** () | **Small RNA sequencing** | **QIAseq miRNA Differential Expression**

1. Double-click on the QIAseq miRNA Differential Expression template 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 10) before clicking **Next**. You can choose either the **mature** (as we do here) or the **seed** expression tables, but not a mix of both.

3. Once again select the QIAseq Small RNA Reference Data Set from the Reference manager (figure 11).

4. In the Differential Expression for miRNA dialog, set the parameters as seen in figure 12. The metatable data is the one you associated with your data in the beginning of the tutorial.

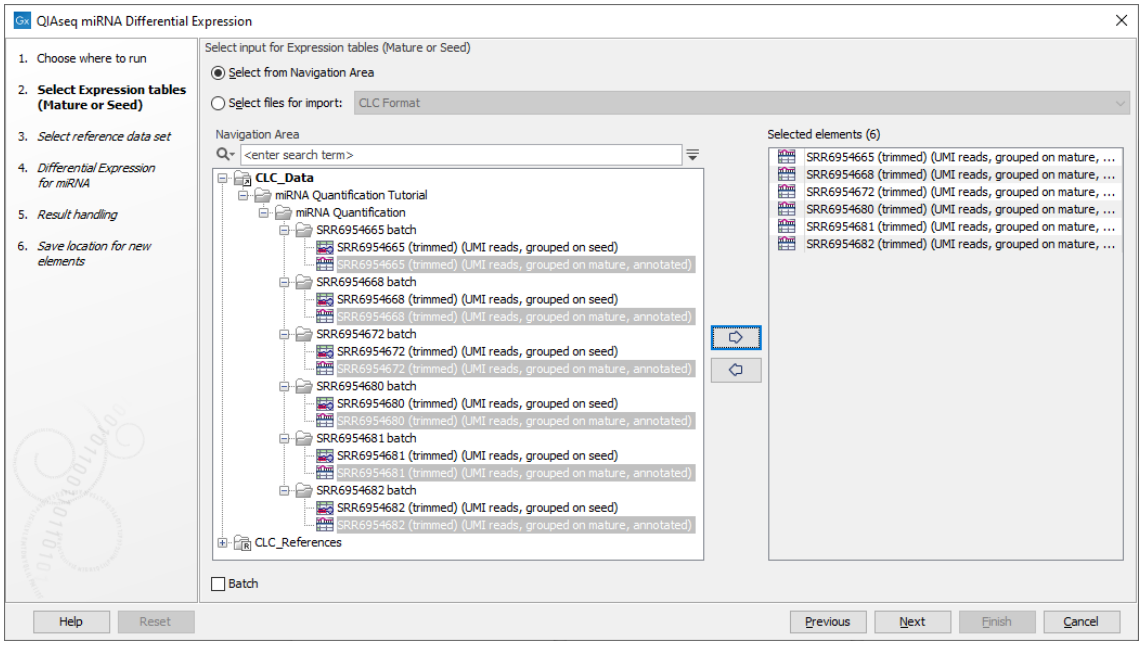


Figure 10: *Select the mature or seed expression tables.*

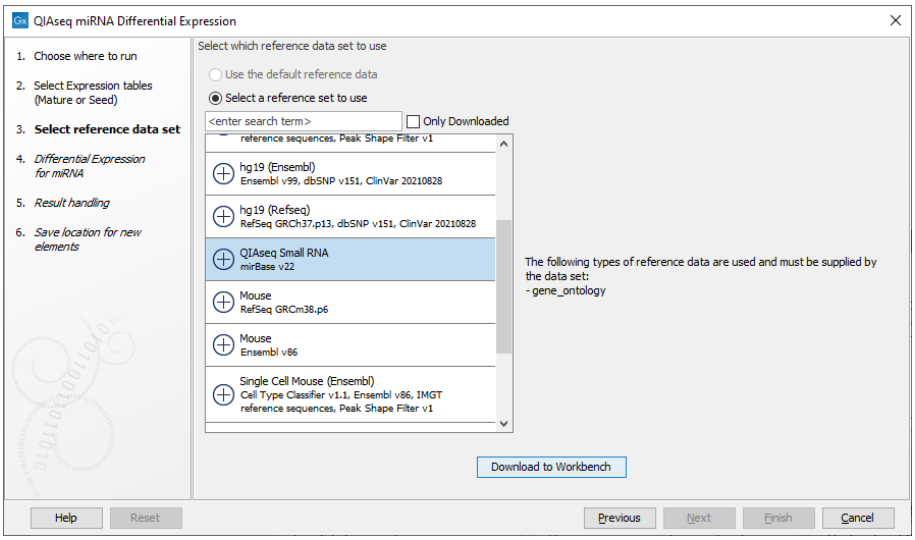


Figure 11: *Select the QIAseq Small RNA reference data set.*

5. Choose to save the results (shown in figure 13) in the tutorial folder.

6. Open the Heat Map for RNA-Seq output (figure 14). 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 15). In this table, the expressed miRNAs that were chosen in the original paper for validation have been highlighted. In our tutorial data, hsa-miR-451a, hsa-miR-486-5p, hsa-miR-363-3p, and hsa-miR-150-5p show significant overexpression in AML relative to ACC as well.

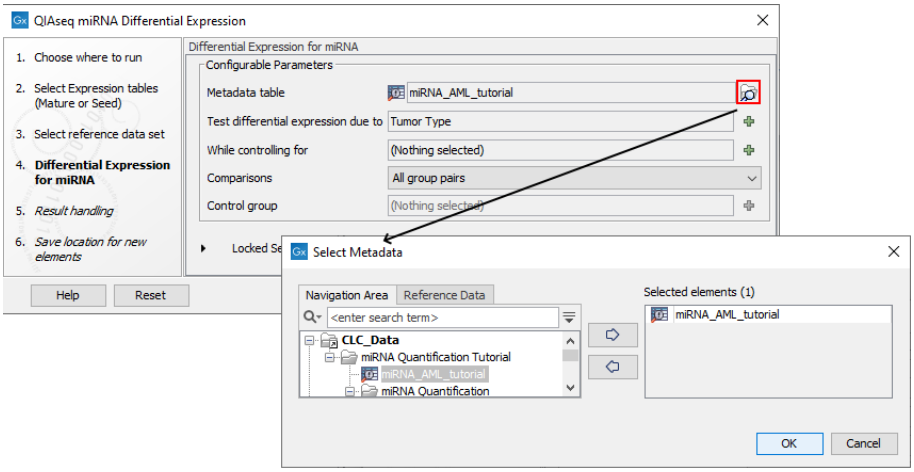


Figure 12: *Parameters for the differential expression analysis.*

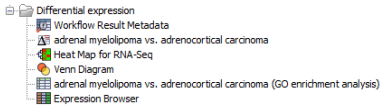


Figure 13: *Outputs of the differential expression analysis.*

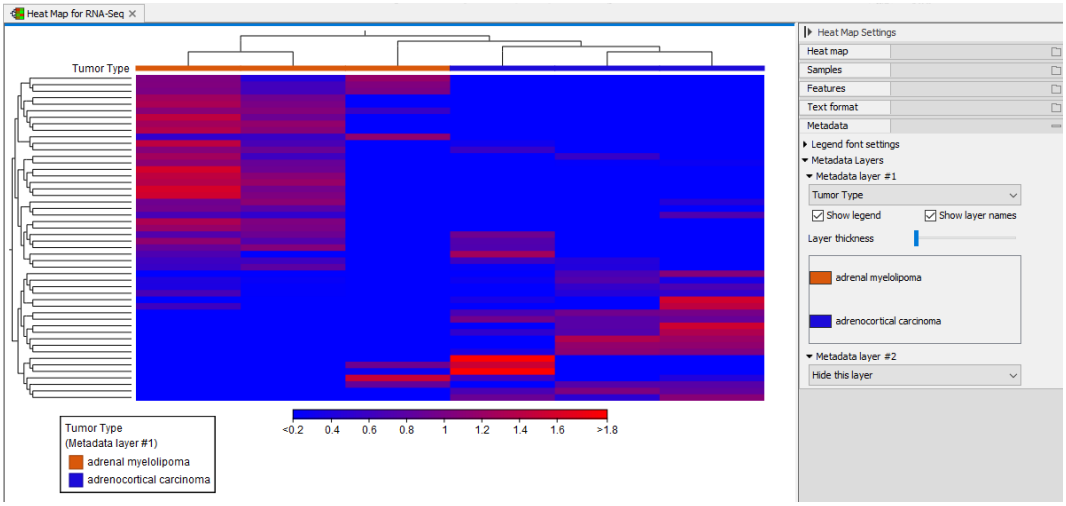
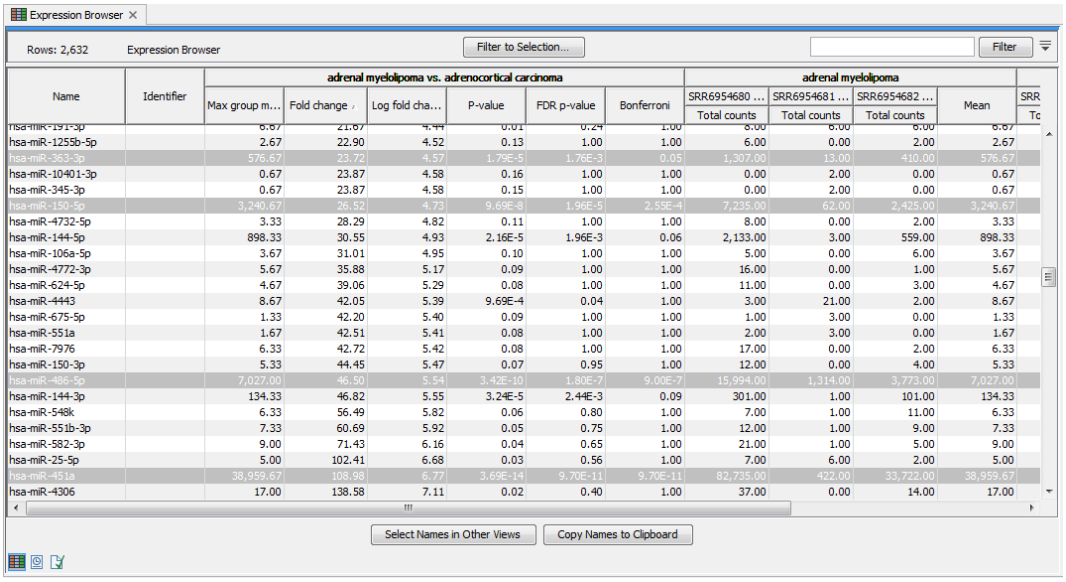


Figure 14: *Heat map for RNA-Seq.*

The Quantify miRNA tool only finds and quantifies the expression of known miRNAs. If you want to dig deeper into how to explore novel miRNAs please have a look here https://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Explore_Novel_miRNAs.html.



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 15: *Expression Browser.*

Bibliography

[Decmann et al., 2018] Decmann, A., Perge, P., Nyiro, G., Darvasi, O., Likï¿½, I., Borka, K., Micsik, T., Tï¿½th, Z., Bancos, I., Pezzani, R., Iacobone, M., Patï¿½cs, A., and Igaz, P. (2018). Microrna expression profiling in adrenal myelolipoma. *Journal of Clinical Endocrinology and Metabolism*, 103(9):3522–3530.