ACSBIOMARKER

Technote-microRNA profiling

MicroRNA profiling

MicroRNAs are a class of small RNAs that regulate post-translational gene expression. MicroRNAs have been implicated in cellular and developmental processes as well as a large number of human diseases. In addition to expression in cells, microRNAs have also been shown to be present in biofluids such as serum, plasma, urine and CSF. Due to their presence in biofluids microRNAs show great potential as non-invasive biomarkers for molecular diagnostics. With our microRNA profiling service we help you obtain quality microRNA profiles that will help you identify and validate novel microRNA biomarkers.

RNA extraction

MicroRNA profiling can be successfully performed on a wide variety of sample types, including FFPE tissue, frozen tissue, cells, exosomes and biofluids (plasma, serum, whole blood). RNA extraction from these samples, especially biofluids, can be challenging and each method requires an optimized approach. For the microRNA profiling service you can either perform the RNA extraction yourself or you can select our *RNA extraction service*. In case you will perform the RNA extraction yourself, we will of course provide guidelines and advice on what method works best for your samples to ensure you obtain high quality RNA.

Hemolysis screening

Hemolysis can contaminate the cell-free microRNA profile of serum and plasma samples. For these sample types we offer a *hemolysis screening service*. A quick and low cost method is to measure oxyhemoglobin absorbance at 414nm by spectrophotometry. Alternatively hemolysis can be screened with a qPCR based method where the ΔΔCq of miR23a-miR451 is applied to identify hemolytic samples (Blondal et al., 2013).

Standardizing input

RNA extraction from biofluids is challenging and total RNA yields are so low that the standard methods for measurement of RNA yield and quality are inappropriate to use. For this reason input amounts for biofluids are standardized according to starting volume and sample quality is assessed by a qPCR-based method. For other sample types that will have a higher RNA yield, concentrations will be determined by nanodrop and a fixed total RNA input will be applied for standardization. RNA quality is also assessed by qPCR.

Assessing sample quality

The quality of RNA is assessed by qPCR through the application and measurement of synthetic spike-in microRNA molecules in combination with endogenous microRNAs. The synthetic spike-ins are added during RNA extraction from biofluids and during cDNA synthesis (independent of sample-type). Applied spike-ins monitor RNA extraction efficiencies as well as the presence of RT and qPCR inhibitors. Biologically relevant endogenous microRNAs are measured as a general sample quality check.

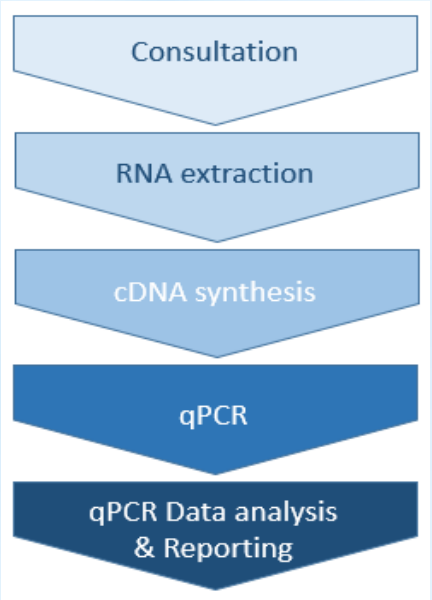
Technote – microRNA profiling

microRNA profiling platform

For our microRNA profiling we apply the QuantaBio microRNA profiling platform in combination with the PerfeCTa SYBRGreen super mix. This platform provides excellent quality qPCR results and allows the profiling of hundreds of microRNAs from a single RT reaction. The platform provides an open flexible assay design and eliminates inefficient stem-loop priming and costly hydrolysis probes. PCR amplicon specificity is verified by melt curve analysis in combination with a positive control sample which is present in each run. Each sample will be measured in triplicate to ensure quality results.

For more information visit the QuantaBio website h*ttp://www.quantabio.com/products/microrna-profiling*

Workflow



qPCR data analysis

At ACS Biomarker we perform a state-of-the-art qPCR data analysis developed in collaboration with the epidemiology department of the AMC. Our analysis method is a PCR efficiency corrected method, which is in many ways superior to the ΔΔCq method. The qPCR analysis consists of data analysis with the LinRegPCR software package. Subsequently interplate correction is performed with qFactorPCR or an interplate corrector. Further data clean-up is performed according to a novel algorithm, which discriminates between valid, invalid and undetectable samples and also includes missing data handling. Lastly, normalization of the data set is performed with the technical normalizers (spike-ins) and endogenous normalizers as selected at the start of each project. For more information on our data handling pipeline check out our *qPCR data analysis technote*.

Data Reporting

After our microRNA profiling service we will deliver an extensive report containing a comprehensive overview of the project, the quality control measurements, raw data, data analysis and all experimental conditions, according to the MIQE guidelines. The final data set can be delivered as excel file or SPSS database file and will be ready for further statistical analysis.

We offer support from start to finish and after delivery of the report our staff is available to answer any remaining questions about the project.

MicroRNA profiling tailored to your research needs and budget

Our microRNA profiling service is known for its high flexibility and can be adapted to your specific research needs and budget. Feel free to contact us to discuss the possibilities.