Service Profile

miRNA Sequencing Service

Robust differential expression analysis along with novel miRNA discovery, enabling you to further your goals

Profiling of miRNAs holds great promise due to their potential use as biomarkers for various diseases. Still, a number of limitations are associated with miRNA profiling. QIAGEN® Genomic Services overcomes these limitations by combining our innovative QIAseq® miRNA-seq technology with decades of technical expertise across a large variety of challenging samples. Extend your in-house resources with the expertise and high quality services you can expect from QIAGEN.

Our all-in-one miRNA Sequencing Service offers the following benefits:

- **End-to-end service:** We take care of every step, from sample preparation to data analysis
- **Highly efficient library preparation:** Bead-based method removing adapter dimers and unwanted RNA species
- **Elimination of PCR duplication and sequencing bias:** Unique Molecular Indices (UMIs) allowing for quantification of single miRNA molecules
- **Ready-to-publish data:** We deliver comprehensive reports and data packages, and provide guidance on the next steps

Partner with us for expert guidance and dedicated service – from Sample to Insight® – for profiling your samples today.

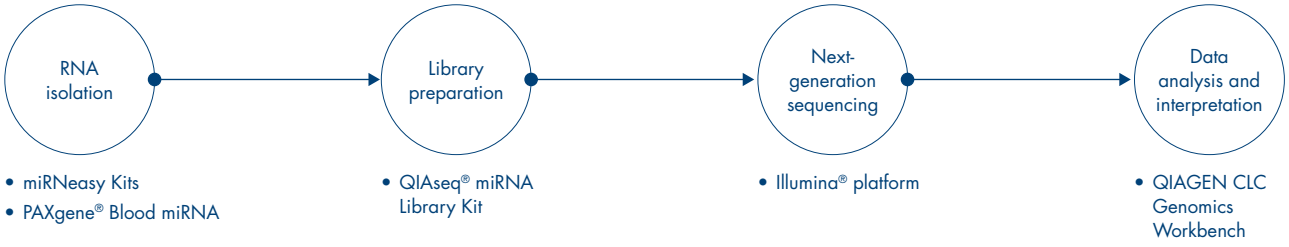

Figure 1. Sample to Insight miRNA Sequencing Service workflow.

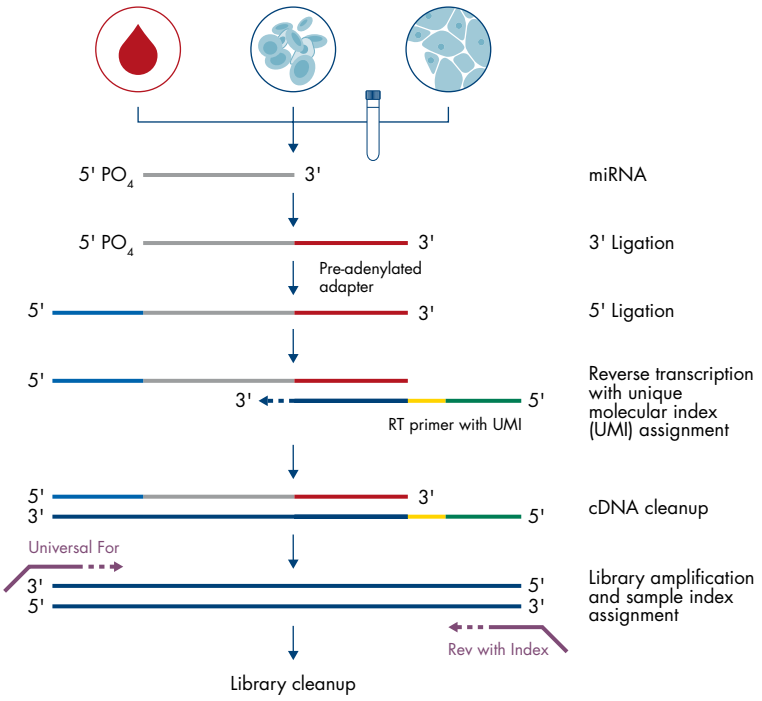

Figure 2. QIAseq miRNA Library Kit workflow. Optimized reaction chemistry enables robust, miRNA-specific libraries while minimizing reaction biases and eliminating adapter dimers. Unique Molecular Indices (UMIs) tag each miRNA at an early stage, eliminating PCR and sequencing bias.

Bioinformatics: a bridge between data and discovery

Novel insights often remain elusive without the right tools and expertise for data analysis and interpretation. QIAGEN Genomic Services uses industry-leading pipelines and best-in-class algorithms to provide you with the answers to your biological questions. The examples provided below show data analysis results including publication-grade graphs and figures which are part of the Genomic Services deliverables.

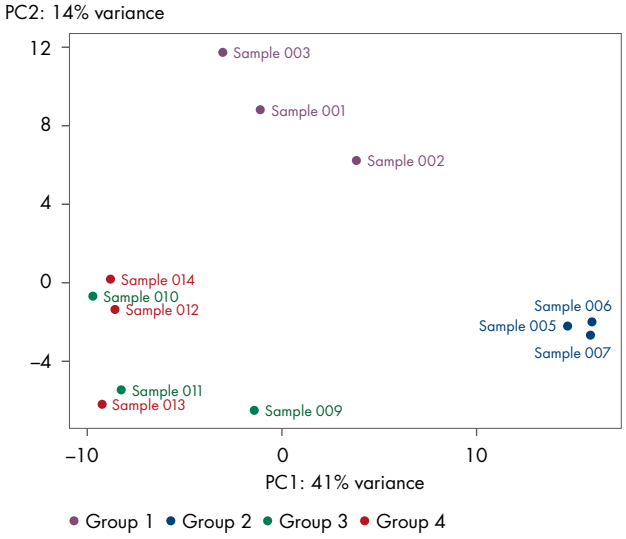

Figure 3. Principal component analysis (PCA) – sample clustering based on the expression profile. The data points that represent the samples are projected onto a two-dimensional plane such that they spread out in the two directions that explain most of the differences. A variance-stabilizing transformation is performed on the raw count matrix, and 500 genes with the highest variance are used to plot the PCA.

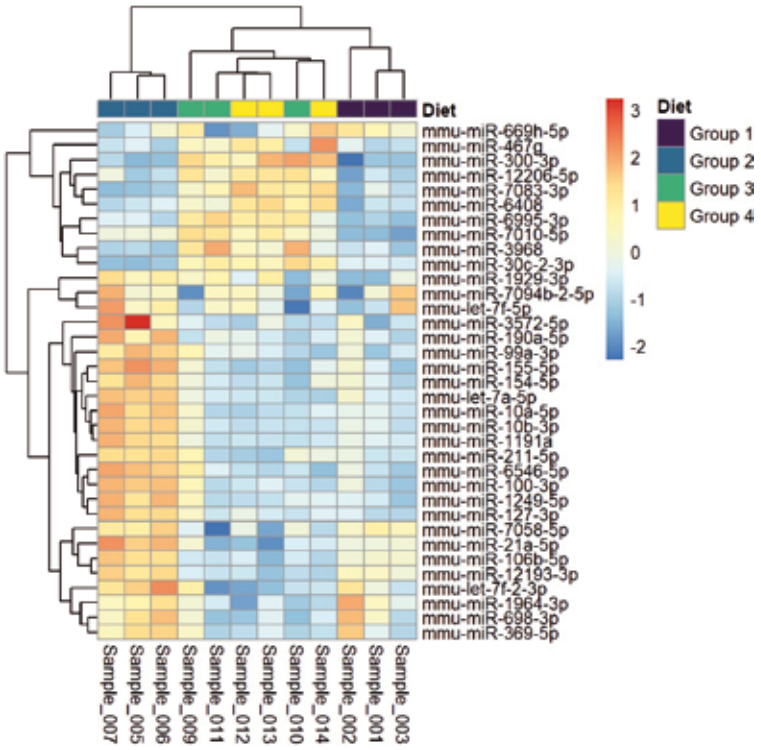

Figure 4. Hierarchical clustering – identification of gene expression patterns. A variance-stabilizing transformation was performed on the raw count matrix, and 35 miRNAs with the highest variance across samples were selected for hierarchical clustering. Each row represents one specific miRNA and each column represents one sample. The color represents the difference of the count value to the row mean.

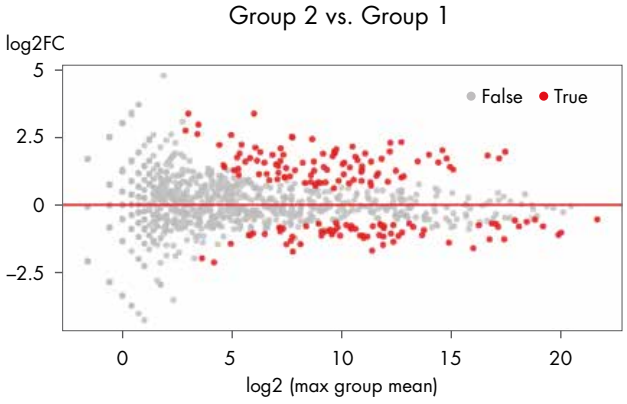

Figure 5. Differential gene expression. For each differential gene expression analysis, the result of the statistical test is represented in a separate MA plot. Each gene's fold-change is plotted against its mean expression among all samples. All significantly differentially expressed miRNAs are marked in red. Significant changes are defined as false discovery rate (FDR) <0.01.

Service specifications

Consultation	Free consultation with an expert to design an experimental setup that best meets your needs.		
Sample requirements	**Sample input**	**Isolation kit**	**Input requirements**
	Customer-isolated RNA	N/A	Minimum: 160 ng total RNA (>10 ng/µl) Recommended: 300 ng total RNA OD_{260}/OD_{280} value >1.6 RIN value >7
	Cells	miRNeasy Mini	Minimum: 2 x 10^6 cells, pelleted and frozen
	Fresh frozen tissue	RNeasy Plus Universal (total RNA including miRNA)	Minimum: 5 mg Maximum: 50 mg
	FFPE	miRNeasy FFPE	Minimum: 2 x 10 µm sections of 250 mm^2 Maximum: 4 x 10 µm sections of 250 mm^2
	Blood (PAXgene)	PAXgene Blood miRNA	Recommended: 1 tube
	Other	Please inquire	
Sample quality control	Fluorescence-based dye for determination of sample concentration Gel electrophoresis for determination of RNA integrity (e.g., RIN value from capillary gel electrophoresis)		
	This is a STOP/GO point where it is possible to omit samples or replace samples before proceeding		
Library preparation and quality control	Library preparation using QIAseq miRNA Library Kit Integrated UMI technology Gel-free, bead-based size selection (15–40 nt) Library quality control by gel electrophoresis to check for the right fragment size and concentration This is a STOP/GO point where it is possible to omit samples before proceeding		
Sequencing parameters	Illumina NextSeq® or Illumina NovaSeq® systems Single-end reads Read length of 75 bp Read depth of 12million reads on average		
Complete data analysis	Raw data	De-multiplexed FASTQ files	FASTQ.GZ
	Raw data quality control	CLC graphical QC report (for each sample)	PDF
		CLC supplementary QC report (for each sample)	Excel
	Filtering and deduplication	UMI report (for each sample) Removal of adapters, low-quality, short sequences and ambiguous nucleotides Deduplication statistics	PDF
	Mapping miRbase (v22) and small RNA databases	miRNA quantification report (for each sample and for combined samples) Mapping rates and annotation statistics Spike-in information included when applicable	Excel
		Raw counts matrix	Excel
		CPM-normalized counts matrix	Excel

Complete data analysis 	Mapping genome (GRCh38 for human) Reads that do not map to miRBase with perfect complementarity or as isomiRs are mapped to the genome (if available). No differential expression analysis is provided on extra annotation.	RNA-seq report	PDF
		Raw counts matrix	Excel
		TPM-normalized counts matrix	Excel
	Unsupervised analysis of miRNA expression	PCA plot	PDF
		Hierarchical clustering and heatmap	PDF
	Differential expression for miRNAs Maximum 10 defined comparisons. Inquire for additional analysis.	Differential expression statistics for each defined comparison Fold-change, $\log_2$ FC, p-value, FDR-corrected p-value, Bonneferoni corrected values	Excel
		MA plot	PDF
	Species supported	All species published in miRbase (v22): http://www.mirbase.org/index.shtml are supported. **Inquire** for other species.	
	Pathway analysis	QIAGEN Ingenuity Pathway Analysis® (IPA®) miRNA-only projects include limited analysis. miRNA combined with whole transcriptome and mRNA data are recommended for full IPA analysis. IPA analysis is available as an add-on; refer to IPA demo report. Supported for human, rat and mouse. **Inquire** for other species.	Various
	Merge data with data from previous projects	**Inquire**	
	Data delivery	Encrypted USB/HDD or cloud delivery	
Final report and consultation 	Final data analysis package contains the following: • Overview of data analysis and algorithms used • Files and tables listed under "Complete data analysis" • Publication-ready figures are typically provided as PDF. **Inquire** for SVG or other formats. Teleconference with QIAGEN scientists to discuss analysis and validation of results. Consultation and support will be provided for 90 days following delivery of data (for data-delivery-only projects) or delivery of data analysis (for data-analysis-inclusive projects). **Inquire** for extended support beyond 90 days.		

Note: Service specifications might be tailored to the needs of the project on a case-by-case basis.

How can we accelerate your research?

Our expert team is looking forward to learning about your research project and designing your customized service with QIAGEN.

Tell us about your project

The QIAGEN® Genomics Service is intended exclusively for research use only (RUO). This service is not intended for the diagnosis, prevention or treatment of a disease.

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