Service Profile

mRNA Sequencing Service

Every step of your mRNA sequencing, quality control and analysis is designed for success

Our mRNA Sequencing Service enriches for poly-A tailed transcripts to increase the sequencing depth for coding mRNAs, which improves the sensitivity to mRNAs expressed at low levels. In addition, the library preparation retains information about which of the two DNA strands was used to transcribe a given RNA. This information provides increased confidence in transcript annotation and enables detection of antisense transcript expression. During data analysis, mitochondrial poly-A tailed transcripts are filtered, as they are considered to be high-abundance sequences.

mRNA sequencing is recommended for discovery work and especially for differential expression analysis. Paired-end sequencing increases the mapping percentage to poorly annotated genomes and makes it possible to identify splice variants with much higher confidence. However, if differential gene expression is the primary goal of your project, we recommend single-end sequencing.

- **End-to-end service**: we take care of every step, from sample preparation to data analysis
- **Full-spectrum solution**: we provide a seamless flow from biomarker discovery to clinical assay development and approval
- **Insightful data analysis**: pathway, upstream regulators and disease analysis of differentially expressed genes can be provided with the industry-leading Ingenuity Pathway Analysis software

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

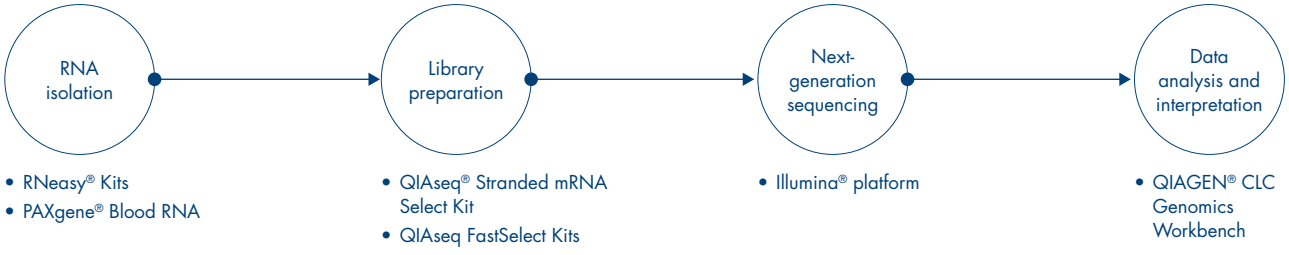

Figure 1. Sample to Insight mRNA Sequencing workflow.

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 use industry-leading pipelines and best-in-class algorithms to provide you with the answers to your biological questions. Below are some examples of data analysis results, including publication-grade graphs and figures, which are part of the Genomic Services deliverables.

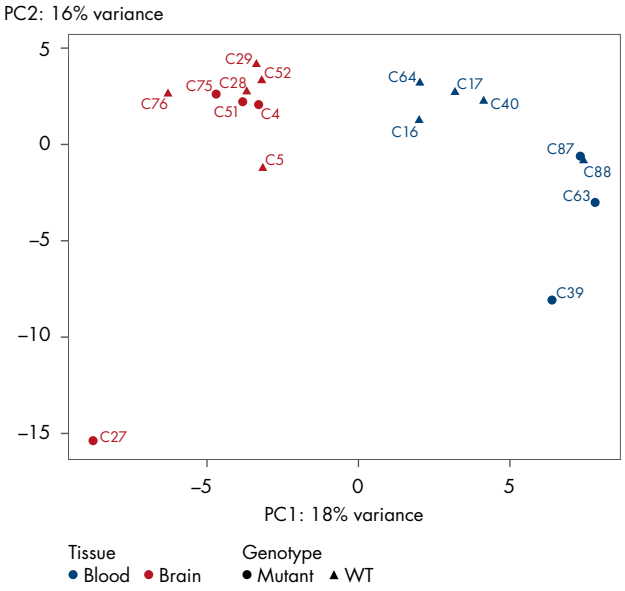

Figure 2. Principal component analysis (PCA) - sample clustering based on the expression profile. The data points that represent the samples are projected onto a two-dimensional plane such that they spread out in the two directions that explain most of the differences. A variance stabilizing transformation is performed on the raw count matrix, and 500 genes with the highest variance are used to plot the PCA.

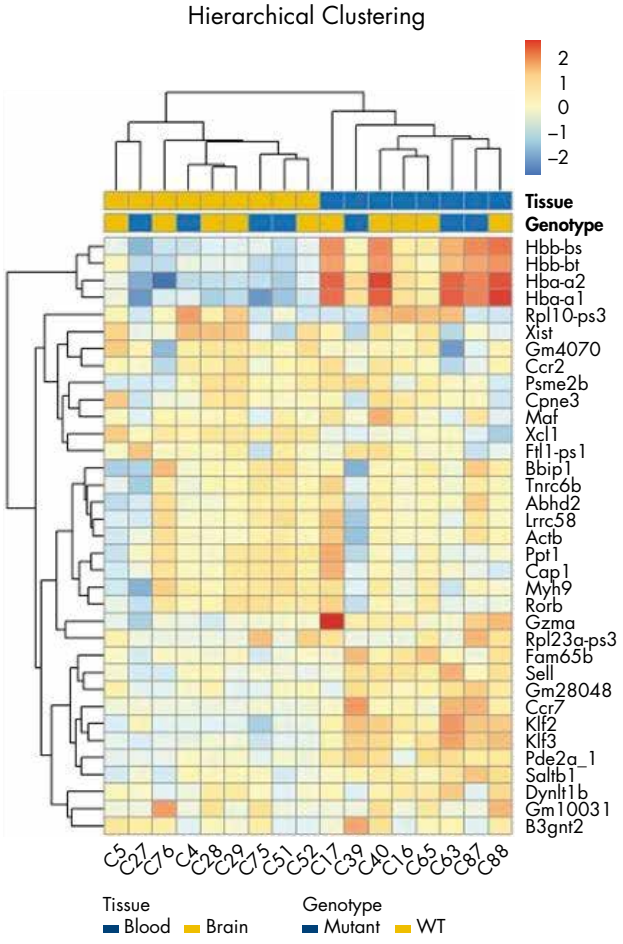

Figure 3. Hierarchical clustering - identification of gene expression patterns. A variance stabilized transformation was performed on the raw count matrix, and 35 genes with the highest variance across samples were selected for hierarchical clustering. Each row represents one gene and each column represents one sample. The color represents the difference of the count value to the row mean.

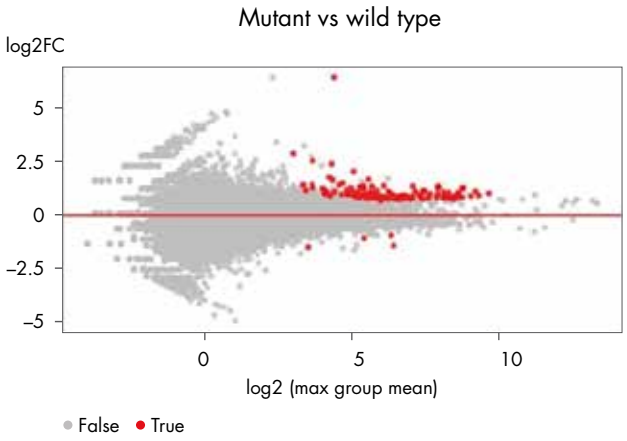

Figure 4. Differential gene expression. For each differential gene expression analysis, the result of the statistical test is represented in a separate MA plot. Each gene's fold-change is plotted against its mean expression among all samples. All significantly differentially expressed genes are marked in red. Significant changes are defined as 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: 200 ng total RNA (>10 ng/µl) Recommended : >500 ng total RNA OD_{260}/OD_{280} Value: >1.6 RIN value: >7
	Cells	RNeasy Plus	Minimum: 2 x 10^6 cells, pelleted and frozen
	Fresh frozen tissue	RNeasy Plus Universal	Minimum: 5 mg Maximum: 50 mg
	FFPE	RNeasy 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 RNA	Recommended: 1 tube
	Other	**Please inquire.** Not suitable for biofluids or exosomes.	
Sample quality control	Fluoresence-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 the QIAseq Stranded mRNA Select Kit for poly-A enrichment Library quality control by gel electrophoresis to check for the right fragment size and concentration Strand-specific library prep 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 or paired-end reads Read length of 75 bp Read depth of 1 x 30 M reads or 2 x 30 M 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
	Data trimming	CLC trim report (for each sample) Removal of adapters, low-quality, short sequences and ambiguous nucleotides	PDF
	Mapping (GRCh38)	RNA-seq feport (for each sample and combined) Read count statistics or mapping rates, fragment counts, distribution of biotypes, transcript coverage	PDF
	Quantification	Raw counts matrix	Excel
		TPM-normalized counts matrix	Excel
	Unsupervised analysis	PCA plot	PDF
		Hierarchical clustering and heatmap	PDF
	Differential expression (for each defined comparison)	Differential expression statistics Fold-change, $\log_2$ FC, p-value, FDR-corrected p-value, Bonneferoni corrected values	Excel
		Volcano plot Maximum 10 defined comparisons. **Inquire** for additional analysis.	PDF
	Pathway analysis	QIAGEN Ingenuity Pathway Analysis® (IPA®) Available as an add-on; refer to IPA demo report. Supported for human, rat and mouse. **Inquire** for other species.	Various
	Species Supported	*Bos Taurus, Caenorhabditis elegans, Canis familiaris, Danio rerio, Drosophilia melanogaster, Equus caballus, Gallus gallus, Homo sapiens, Mus musculus, Oryza satvia, Pan troglodytes, Rattus norvegicus, Sus scrofa.* **Inquire** for other species.	
	Merge data with data from previous projects	**Inquire**	
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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