Gene expression | Total transcriptome | Ultra low-input | FFPE tissue | Single cell sequencing

mRNA Gene-expression analysis

(mRNA expression based on poly-A selection)

Sequencing of all protein-coding genes

Through their poly-A tails, all messenger RNA (mRNA) can be captured and sequenced specifically for mRNA gene expression analysis. This provides an affordable approach to give insight into differential gene expression between groups of samples, such as various treatments, time-points, or disease versus control samples. RNA from different types of tissues and body fluids can be assessed. Specifically, for whole blood analysis, we offer globin reduction that removes the globin transcripts originating from erythrocytes from your samples. This reduces the sequencing capacity required for your sample by 30-40% and lowers affiliated costs.

Input material

Isolated total RNA

Isolated sample requirements

- Optimal total RNA quantity: ≥ 1000 ng / sample
- Minimal RNA quantity: 200 ng / sample
- Minimal volume of 25 µl / sample
- Required sample quality: RIN ≥ 7 / RQN ≥ 6
- OD 260/230 ratio in the range 1.8-2.2
- OD 260/280 ratio in the range 1.8-2.2

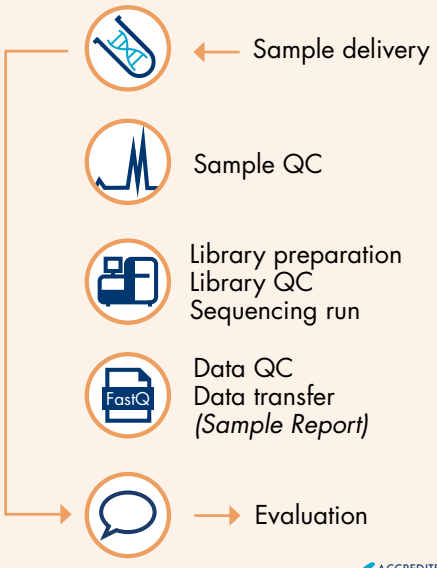
Sequencing on Illumina NovaSeq (PE 150)

- Standard read depth 20M PE reads/ sample or above depending on your needs
- Unique Molecular Identifier tags

Deliverables

- FastQ files
- Quality score (Q30) ≥ 80%
- Optional data analysis with comprehensive report

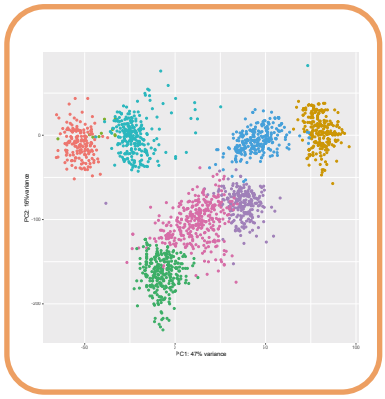
NGS Laboratory workflow



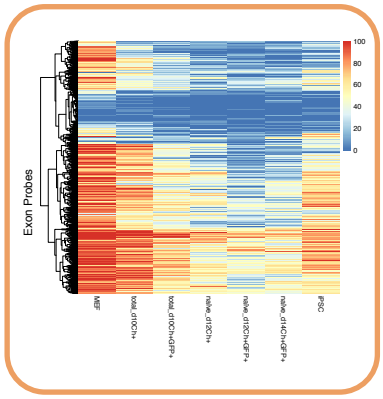
ACCREDITED ISO/IEC 17025 L518 By RvA

Sample Purity

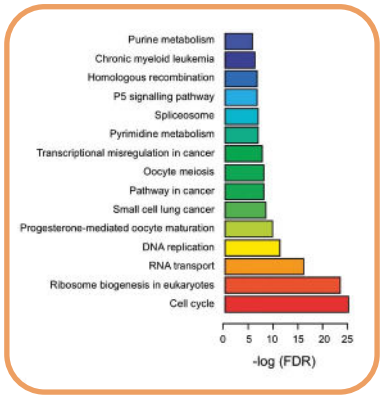
The purity of the sample is of high importance when determining the input and quality required to proceed with the library preparation. When measuring concentrations with nanodrop, the presence of compounds like guanidium isothiocyanate and phenol can greatly inflate the concentrations, giving a much higher concentration than the actual value. These compounds as well as others, like EDTA and isopropanol, also greatly influence the effectiveness of the



Principal components analysis



Heatmap



Pathway analysis

sample preparation. All these compounds can be found in the samples due to the isolation process and the reagents involved, especially during the final elution step.
Refer to the following articles for more details on the influence of these contaminants.

- *https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001249*
- *https://community.nanoporetech.com/contaminants (the Ligation Seq Kit library prep efficiency graphs are comparable to our prep method)*

Committed to your project

Data quality guarantee

Depth of coverage, base quality and data quality are essential metrics to evaluate the quality of your NGS data.

Reads of unique transcripts

A known challenge of NGS sample prep is the formation of PCR duplicates (inversely related to the amount of sample input). Our RNA NGS service includes Unique Molecular Identifiers (UMIs) that ensure the ability to identify these PCR-artifacts and hence allow the read representation of truly unique transcripts.

Results

We have dedicated data-analysis pipelines to provide you with the output figures to best represent your data, for every option from microRNAs to long non-coding RNAs and from high-throughput screening methods to delicate single-cell sequencing.

Data analysis options

Our data analysis report provides multiple visualization options (see frame above) to make data easily comprehensible and useable for decision makers. The report summarizes the most relevant information, with additional technical details in appendices or individual sample reports. It is based on many years of experience working with customers and operating under a stringent quality system.

Robust industry-standard methods are used to determine gene expression levels and identify differentially expressed genes between biological conditions.
The results can be viewed as summary tables, individual gene lists, or heatmaps.
Read mappings can be visualized using many intuitive graphical user interfaces. Multiple levels of quality controls ensure read integrity and biological plausibility of the results.

Biological insights

The biological insights that can be inferred from your data include:

- *de novo* transcriptome assembly
- Discovery of transcripts and variants
- Differential expression analysis of genes, transcript variants, and exons (alternative splicing)
- Analysis of gene fusions and trans-splicing events
- Gene regulatory networks, signaling pathways and networks, and gene enrichments
- Host/pathogen interactions or xenografts

Custom analysis

Custom bioinformatics can be performed allowing more in-depth mining of your data set. Functional gene information mining, gene enrichment set, gene ontologies may be additionally provided when required.

About GenomeScan

As an ISO-accredited leading Dutch Next Generation Sequencing service provider, GenomeScan develops customizable NGS solutions for pharmaceutical and biotech companies, healthcare providers and academic institutions. By providing state-of-the-art tools to analyze genetic disorders fast, affordably, and effectively, GenomeScan fosters innovation through partnership with medical centers and research laboratories.

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