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

Ultra-low Input Transcriptomics

Robust RNA gene expression analysis from single cell level

Gene-expression analysis becomes more specific when small populations are screened. Samples that contain multiple cell types show much variation, mostly reflecting the sample-composition. Selecting cell clusters that differ solely in those aspects that you want to study are easier to interpret and allows you to detect significant changes in gene-expression for low abundant transcripts.

Discovery of biomarkers and transcription-factors has never been so easy. The low-input transcriptomics method is applicable for both high and low quality RNA and is successfully validated for challenging samples. Therefore, you can rely on uniform transcript coverage, regardless of input amount or sample type.

Input material

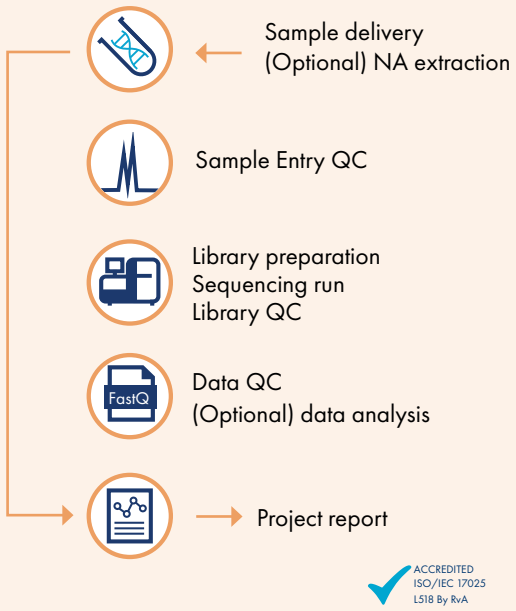
Isolated total RNA

Isolated Sample Requirements
- Optimal total RNA quantity: 30 ng / sample
- Minimal RNA quantity: ≥ 0.25 ng / sample
- Minimal volume: 25 µl / sample
- Minimal quality (FFPE): DV200 > 25%

Sequencing on Illumina NovaSeq (PE 150)
- Standard sequencing read depth 30M PE reads / sample
- Unique Molecular Identifier tags

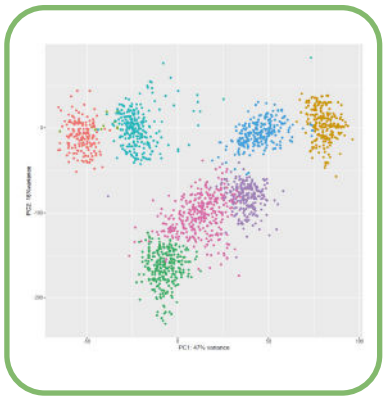
Deliverables
- FastQ files
- Quality score Q30 of ≥ 80%
- Project Report
- Optional data analysis with comprehensive report

NGS Laboratory workflow

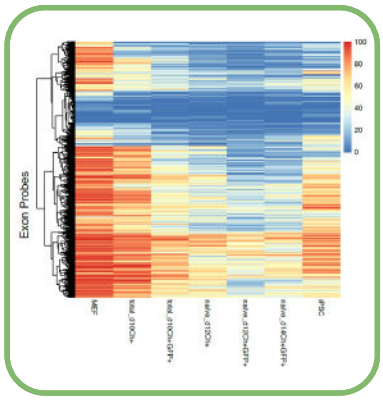


Sample Purity

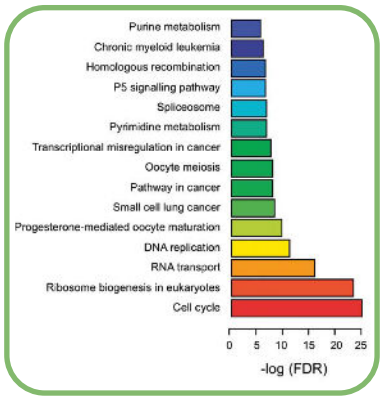
The purity of the sample is of high importance when determining the input and quality required to proceed with the library preparation. When measuring concentrations with nanodrop, the presence of compounds like guanidium isothiocyanate and phenol can greatly inflate the concentrations, giving a much higher concentration than the actual value. These compounds as well as others, like EDTA and isopropanol, also greatly influence the effectiveness of the sample preparation. All these compounds can be found in the samples due to the isolation process and the reagents involved, especially during



Principal components analysis



Heatmap



Pathway analysis

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.

Publication ready 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.

Deliverables

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 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 that are available in the public domain. Multiple levels of quality controls ensure read integrity and biological plausibility of the results.

Biological Insights

- 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 solutions

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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