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

Total Transcriptome

Sequencing of all protein-coding genes

Total* transcriptomic sequencing is not restricted to messenger RNA, but involves sequencing of most RNA species, including long non-coding RNA (lncRNA). A ribosomal RNA (rRNA) depletion step is performed to remove the 18S and 28S ribosomal subunits that make up 60-90% of all RNA species present in your sample, allowing for deeper sequencing of your RNAs of interest.

This approach also tolerates the use of lower quality RNA (degraded RNA) for sequencing. Known causes for RNA degradation include FFPE-fixation and lasercapture methods. Our scientists have set up validated procedures to analyze these challenging samples. These include clinical-grade sample isolation from FFPE material and tailored data-analysis.

Input material

Isolated total RNA

Isolated Sample Requirements

- Optimal total RNA quantity: ≥ 500 ng / sample
- Minimal RNA quantity: 100 ng / sample
- Minimal volume: 25 µl / sample
- Quality: Moderate degradation is acceptable (based on the fragment profile) if sufficient RNA is available for library preparation.
- FFPE possible with a DV200 of > 25% and ≥ 0.25 ng input

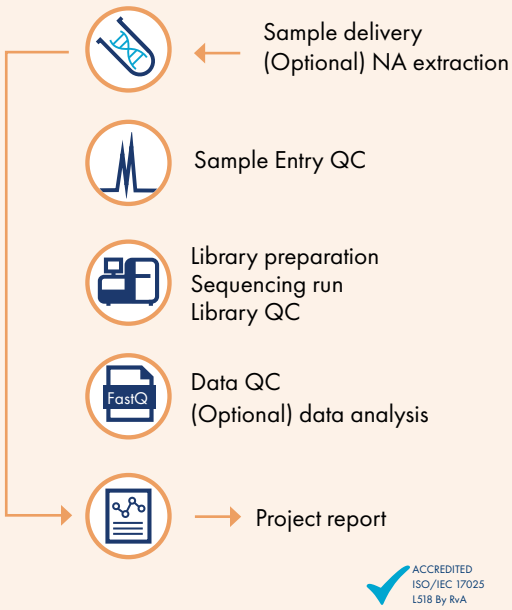
Sequencing on Illumina NovaSeq (PE 150)

- Standard sequencing read depth 30M PE reads / sample
- Removal of Globin RNA possible
- Unique Molecular Identifier tags

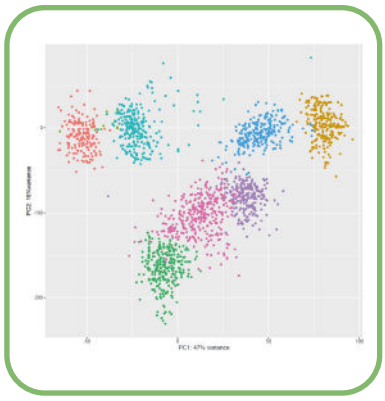
Deliverables

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

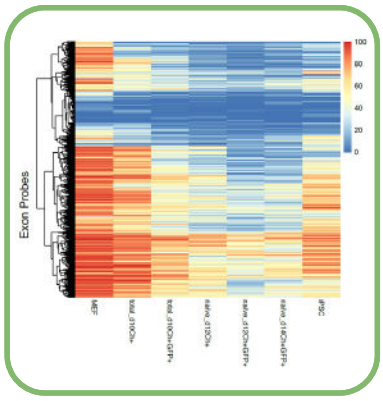
NGS Laboratory workflow



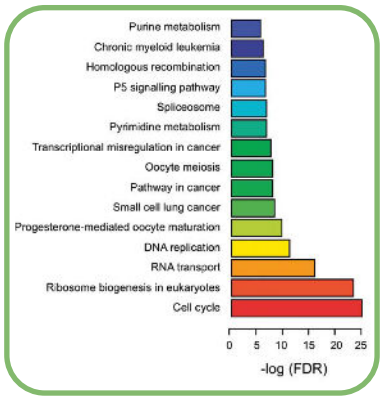
** This service is not suitable for microRNAs and other small RNAs (noncoding RNAs, sncRNA). RNA molecules less than ~200 nucleotides in length are excluded.*



Principal components analysis



Heatmap



Pathway analysis

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 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 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

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 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.

genomescan.nl

+31 71 5681050

info@genomescan.nl

GUIDO02, version 12, Authorized by QA