# Sequencing Services

**Catalogue of Services**

**Using modern high-throughput sequencing methods, hundreds of thousands of genomic regions can be analyzed in parallel within a very short time and with very high sensitivity.**
Next generation sequencing, or high-throughput sequencing, today allows the genetic analysis of the entire genome or transcriptome, a genetic profiling of a person or organism. In addition to whole genome or transcriptome sequencing, methods such as exome sequencing are an alternative to cover all coding regions. Targeted sequencing allows the investigation of disease-associated genes or addresses specific questions. The spectrum of methods is broad and continues to evolve. The possibility of parallel high-throughput sequencing of the entire genome opens up many possibilities in a reasonable time.

**Starting material:**
- Blood or bone marrow
- Isolated human cells in an appropriate buffer
- Extracted DNA or RNA of high quality
- Prepared libraries
- Pools ready for sequencing

**Sample preparation:**
- Isolation of white blood cells from bone marrow and peripheral blood
- Extraction of genomic DNA and RNA from human cells stored in an appropriate buffer

**Next generation sequencing:**
- Single amplicon sequencing of a defined set of human genes
- PanelSeq – Targeted gene panel sequencing for a variety of available panels as well as customized ones
- The enrichment of the targeted genes follows the library prep by hybridization capture
- WES - Whole exome sequencing (human)
- WGS - Whole genome sequencing
- WTS - Whole transcriptome sequencing (RNA-Seq)
- Illumina Sequencing-by-synthesis on MiSeq or NovaSeq instruments

**Data analysis:**
- Demultiplexing
- Alignment
- Variant Calling
- Interpretation

**Data transfer:**
- Data will be provided as report, excel file or by a link for download

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## Details:

### Sample preparation

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|---|---|
| O Isolation and enrichment of white blood cells | Enrichment of white blood cells / leukocytes by lysis of the erythrocytes. Subsequently, cells (5 million each) are aliquoted in RLT buffer and can be stored at -80°C. |
| O DNA extraction from cells | DNA extraction from cells is performed using the Roche MagNA Pure System. The extracted DNA is adjusted to 25ng/µl in190µl maximum. The storage takes place in tubes with 2D barcode in the Liconic freezing system. |
| O RNA extraction from cells | RNA extraction from cells is performed using the Roche MagNA Pure System. The quality of the RNA is checked by determination of the concentration and the RIN value. The extracted RNA is stored at -80°C in tubes with 2D barcode in a Liconic freezing system. |
| O cDNA synthesis | The RNA is then reverse transcribed (RT) using VILO IV transcriptase. RNA of an aliquot/asservat is completely used for RT. The obtained cDNA (50µl) is stored at -20°C in tubes with 2D barcode in a Liconic freezing system. |
| O Storage of cells | Storage of aliquoted leukocytes at -80°C. One aliquot contains 5 million cells in RLT buffer. The storage takes place in Fluidx tubes identified by a 2D barcode at the bottom of the tube in the Liconic freezing system. The unit of account is the number of tubes/year. The assignment of the sample to the barcode will be announced by MLL when the material is returned. |
| O Storage of nucleic acid (DNA/RNA/cDNA) | The DNA/RNA/cDNA is stored at the appropriate temperature in tubes with 2D barcodes in a Liconic freezing system. DNA and cDNA are stored at -20°C, RNA at -80°C. The storage takes place in Fluidx tubes identified by a 2D barcode at the bottom of the tube in the Liconic freezing system. The unit of account is the number of tubes/year. The assignment of the sample to the barcode will be announced by MLL when the material is returned. |

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## Library preparation

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|---|---|
| O Library prep for enrichment (TruSeq) | The library prep for enrichment is performed with the Illumina TruSeq DNA Exome Kit using Unique Dual Indices (UDI). The DNA is fragmented to a length of 150bp using the Covaris. The starting material is 150ng DNA. A quality check of the starting DNA is necessary for FFPE or Liquid Biopsy DNA, as it may not have to be fragmented. |
| O Library prep for enrichment (Nextera Flex) | The library prep for enrichment is performed with the Illumina Nextera Flex for Enrichment Kit using Unique Dual Indices (UDI). The DNA is tagmented enzymatically in 180bp fragments in the protocol. 100ng DNA with sufficient fragment length is required as starting material (quality check required for FFPE DNA). |
| O Hybridization capture based enrichment (gene panel) | The DNA target regions are enriched using the IDT Hybridization Capture Protocol and corresponding lockdown gene panels. |
| O Library prep for WGS (TruSeq, PCR free) | The library prep for Whole Genome Sequencing (WGS) is performed with the Illumina TruSeq DNA PCR-Free Kit using Unique Dual Indices (UDI). The DNA is fragmented to a length of 300bp using the Covaris. The starting material is 1µg high-molecular DNA. |
| O Library prep for WGS with limited material (TruSeq) | The library prep for Whole Genome Sequencing (WGS) is performed with the Illumina TruSeq Nano DNA Kit using Unique Dual Indices (UDI). The DNA is fragmented to a length of 300bp using the Covaris. The starting material is 100ng high-molecular DNA. |
| O Library Prep for WTS (TruSeq, total RNA) | The library prep for Whole Transcriptome Sequencing (WTS) is done with the Illumina TruSeq Stranded Total RNA Kit using Unique Dual Indices (UDI). The starting material is 250ng RNA with a RIN value >5. |
| O Library prep for RNA exome sequencing (TruSeq) | The library prep for RNA exom sequencing is performed with the Illumina TruSeq RNA Exome Kit using Unique Dual Indices (UDI). The starting material is 250ng RNA. FFPE RNA can also be used with this protocol, which usually shows a significantly lower RIN value (RIN <7). The enrichment of the exonic regions is done with the xGen Exome Research Panel (IDT). |

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## Sequencing

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| O Sequencing gene-panel | All targeted NGS analyses will be performed with a mean coverage above 1,000-fold and a sensitivity of 3%. Result reports will be provided as an Excel file providing read depth (mean coverage), DNA annotation, protein annotation, allele frequency [%] of variants, as well as interpretation of the variants in terms of SNP, variant or somatic mutation. |
| O Sequencing WES (100x) | Sequencing of a WES library with a mean target coverage of 100x on an Illumina NovaSeq 6000 system. |
| O Sequencing WGS (30x-90x) | Sequencing of a WGS library with a mean target coverage of 30 - 90x on an Illumina NovaSeq 6000 system. |
| O Sequencing WTS (50 M reads) | Sequencing of a RNA exome library with a target read count of 50 million on an Illumina NovaSeq 6000 system. |
| O Sequencing per cluster/ read (Mio) | Sequencing of a library/pool based on the targeted readout. The specifications are based on the target read number/cluster passed filter. Sequencing is performed on an Illumina NovaSeq 6000 or MiSeq system. TruSeq or Nextera adaptors are required, if not a complete flow cell or lane is requested. |
| O Sequencing per Gb output | Sequencing of a library/pool based on the targeted Output [Gb]. Sequencing is performed on an Illumina NovaSeq 6000 or MiSeq system. TruSeq or Nextera adaptors are required, if not a complete flow cell or lane is requested. |

## Flow Cells

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| O MiSeq instrument | O NovaSeq instrument (SP, S1, S2, S4) |
| | O Lane of NovaSeq S2 or S4 flow cell |

## Data analysis

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| O Alignment WGS data | Map read data (fastq) to the human reference genome (GRCh37 (hg19), optionally GRCh38) using Isaac aligner, including PCR duplicate marking and indel realignment. Output is a BAM file. |
| O Alignment RNA Seq data | Map read data (fastq) to the human reference genome (hg19 default, hg38 optionally) using STAR aligner and FPKM estimation of reference genes and transcripts using Cufflinks 2 (Salmon optionally). |

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| O Tumor/Normal Pipeline for variant calling (SNV/SV/CNV) | BAM (GRCh37, GRCh38) used to detect somatic variants from tumor and matched normal pair using Strelka2 for SNV and small indels, Manta for stuctural variant calling and CANVAS for CNV. Output is a VCF. |
| O Tumor only Pipeline for variant calling (SNV/SV/CNV) | BAM (GRCh37, GRCh38) used to detect somatic variants from tumor-only sequencing using Isaac Variant caller for SNV and small indels, Manta for stuctural variant calling and CANVAS for CNV. Output is a VCF. |
| O Expression analysis | STAR Aligner BAM is used to perform differential expression analysis of reference genes with DESeq2. Control samples needed. |
| O Variant calling: fusion transcripts and SNV | BAM (hg19, hg38) created by STAR aligner. Fusions are called with Arriba, Manta and STAR-Fusion. Fusions called by at least two callers are reported. PE reads required for fusion detection. Additionally Isaac Variant caller is used for SNV and small indel detection. |
| O Annotation of SNV with public data bases | Annotation of VCF file with Nirvana engine using the following sources: VEP, ClinVar, COSMIC, dbSNP, gnomAD, DGV. Outputs VCF, including alignment and variant calling metrics. |
| O Manual annotation of SNV by MLL pipeline | Manual annotation of variants using MLL routine diagnostics workflow with variant classification. Output is a report/excel file. |

**Data transfer**

Depending on the requested data, data will be provided as report, excel file or by a link for download.