ALMAC

Partnering to Advance Human Health

Whole Exome Sequencing (WES) Data generation service

Research Use Only

Almac WES data generation service

A high quality, robust Next Generation Sequencing (NGS) solution built on Agilent's chemistry. Almac's wet lab optimised workflow generates comprehensive WES data from multiple sample types, including more challenging Formalin Fixed Paraffin Embedded (FFPE) material. The platform covers 99.7% of coding exons with readily interpretable raw sequencing data for use in biomarker discovery and retrospective clinical investigation.

Technical specification:

Platform	
Intended Use	Research Use Only (RUO) for biomarker discovery and retrospective clinical investigation.
Technology	NGS Technology - Illumina NovaSeq 6000, NextSeq 500 and NextSeq 550.
Type	Core laboratory service offering for WES data generation with associated QC report.
Variants	Small variants (SNPs and indels) across gene targets, including all pathogenic variants in genes listed in ACMG guidelines for secondary findings.
Sample requirements	
Tissue type	Optimised for Formalin Fixed Paraffin Embedded (FFPE) tissue. Also compatible with other sample types including Fresh Frozen (FF) tissue & blood.
Recommended tissue requirements	≥ 1mm^3 tissue (macrodissection for oncology research with 10% minimum viable tumour cells).
Input material requirements	100ng DNA from FF or blood samples; 100ng amplifiable DNA from FFPE sample.
Workflow	
Nucleic acid extraction	Optimised DNA extraction protocol based on QIAamp DNA FFPE Tissue Kit or dual DNA & RNA extraction protocol using Qiagen AllPrep DNA/RNA FFPE kit.
Library preparation	Agilent SureSelect^{XT} Low Input Target Enrichment System, optimised for FFPE material.
Library capture	Agilent Human All Exon V7.
Platform performance	
Coverage	100x average on target coverage across cohort (sample quality dependent).
Sensitivity & specificity	Capable of detecting somatic mutations as low as 5% variant allele frequency.
Quality control (QC)	
QC assessment	Comprehensive QC assessment of data including: • Sample & Library QC: DNA input, pre- & post-library capture quality & quantity. • Sequencing Run QC: pre-alignement FastQC metrics. • Post Sequencing & Alignment QC: PICARD Hybrid Selection metrics.
Platform performance	
TAT	For batch retrospective testing - turnaround time (TAT) agreed on a per project basis.
Reporting	
Raw data provided	FASTQ files.
QC Report	Excel compatible report, detailing lab, pre & post alignment data. HTML interactive report (MultiQC) allowing simultaneous assessment of sequence QC data from multiple samples.
Bioinformatics applications	Pre and post alignment QC pipelines hosted on Illumina Basespace Sequence Hub and DNAnexus. Further downstream analysis & reporting can be facilitated by Almac Diagnostic Services Bioinformatics Team if required, at an additional cost.
Added value	
Added value to client	Optimised dual DNA & RNA extraction protocol for FFPE tissue, enables generation of WES & RNAseq data from a single sample.

Key benefits:

- **QC guarantee of 100X mean target coverage** across sample cohort ensures comprehensive downstream analysis and data interpretation.
- **Protocol optimised for challenging samples** including FFPE material.
- **Dual DNA & RNA extraction protocol** enables generation of WES & and RNAseq data from a single sample.
- A comprehensive variant detection **platform, covering 213,994 coding exons**.
- **Machine-learning based bait design** of SureSelect Human All Exon V7 ensures superb streamlined coverage of variants across exons.
- **Interactive QC report (MultiQC)** enables rapid and comprehensive sample quality interrogation across the cohort.
- **Raw Sequence data** delivered in FASTQ format, compatible with the majority of bioinformatics pipelines.

Performance validation:

To assess the robustness and performance of Almac's WES Data Generation Platform, we performed analytical validation using Agilent's Clinical Research Exome V2 (CREV2) panel.

1. Accuracy study

WES data generated at Almac on the CREV2 panel using 100 ng of amplifiable DNA isolated from 40 FFPE samples (8 samples each for breast, ovarian, colorectal, prostate, lung cancer) were compared to the same samples processed at an independent service provider using a different high-quality WES technology. Across all samples processed at Almac, the average on target coverage was 154X and average duplication rates 20%. Data demonstrated overall agreement at 99.99% (CI: 99.9957-99.9958):

Metric	Estimate	Confidence Interval
Overall agreement	99.9957	99.9957 - 99.9958
Positive percent agreement	97.3683	97.3425 - 97.3939
Negative percent agreement	99.9979	99.9978 - 99.9979

Conclusion - Highly accurate WES data generation

2. Precision study

WES data was generated at Almac on the CREV2 panel using 100ng of amplifiable DNA isolated from 3 FFPE cancer samples (breast, colorectal, lung) and 2 control samples. Samples were processed in duplicate (repeatability), performed over 3 runs (repeatability), performed using 3 operators (reproducibility) and using two independent reagent lots (reproducibility). Across samples, repeatability and reproducibility estimates were greater than 99% for insertions, deletions and SNPs:

Variant Type	Repeatability	Batch 1	Batch 2	Operator 1	Operator 2	Operator 3	Reproducibility
SNP	99.93	99.89	99.89	99.9	99.91	99.9	99.87
Ins	99.93	99.89	99.89	99.91	99.91	99.9	99.87
Del	99.94	99.91	99.91	99.92	99.93	99.92	99.9
Combined	99.9	99.86	99.86	99.87	99.88	99.87	99.84

Conclusion - Highly reproducible and repeatable WES data generation

Almac Diagnostic Services expertise:

- More than 15 years' experience in working with challenging sample types, including FFPE.
- High quality service with robust quality control and data assurance.
- Data analysis and interpretation services available for customised investigation of data.
- Your study designed, managed & performed by PhD level scientists in our CLIA & CAP accredited laboratory.

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GET IN TOUCH

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