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FastTrack Whole-Genome Sequencing Services

Providing personalized customer service, generating industry-leading data analysis, and delivering rapid turnaround times for whole-genome sequencing.

Highlights

- Personalized Service and Support Project managers guide researchers through the sequencing process, answer questions, and communicate updates
- Trusted Expertise Industry-leading technology, experienced personnel, and extensive quality control deliver highly accurate data
- Robust and Scalable Process High-throughput automation and an integrated laboratory information management system ensure scalability to manage large studies
- Rapid Turnaround Time
 Integrated sample-to-data solutions provide fast and reliable
 whole-genome sequencing services

Introduction

Illumina FastTrack Services provide whole human genome sequencing services for various research applications. Using sequencing by synthesis (SBS) chemistry—the most widely adopted next-generation sequencing technology—these sample-to-data solutions deliver highly accurate results to provide researchers with a complete view of the human genome. Each researcher receives personalized attention and support throughout a sequencing project. FastTrack Services use automated and traceable processes to preserve data integrity, enabling researchers to focus on the next discovery.

Personalized Service and Support

The FastTrack Services team includes expert scientists, project managers, and laboratory personnel with years of genomics experience. Committed to collaboration, Illumina project managers are available at any time during a project to answer questions, provide guidance, and communicate updates. Project managers track samples throughout the sequencing process, and scientific experts monitor sequencing runs to make sure that the highest data quality is delivered.

Proven Technology

FastTrack Services use SBS chemistry, a reversible terminator–based method that enables massively parallel sequencing and delivers the most accurate human genomic data in the industry. The high data quality results in few false positives and false negatives, so that researchers can be confident in the accuracy of their sequencing data. By coupling the latest innovations in SBS with integrated solutions, FastTrack Services deliver whole-genome sequencing data quickly.



Sequencing Process

FastTrack Services include Whole-Genome Sequencing and Cancer Analysis Services, which use an automated, high-throughput process to achieve optimal sequencing results. From sample receipt through data analysis, all samples are treated with care and focused attention, and are tracked with an integrated laboratory information management system (LIMS) for positive sample tracking. Multiple quality control (QC) steps occur throughout the process to provide high confidence in the sequencing results (Figure 1). If a sample does not meet the quality requirements at any QC step, the sample is evaluated by a staff scientist and the project manager contacts the researcher to discuss available options.

Sample Submission

At the beginning of each sequencing project, an Illumina project manager contacts and guides the researcher through each step of the service process. During this collaborative first step, the project manager also answers questions, addresses concerns, and provides updates regarding the FastTrack Services sequencing workflow and analysis pipeline. When samples are ready for sequencing, the project manager sends the researcher barcoded plates and detailed guidelines for sample preparation and submission. The project manager also provides guidance about plate layout and safe shipping procedures to maintain sample integrity during transit.

Library Preparation

After arrival and visual inspection, each sample is quantified using an automated PicoGreen assay. This assay enables FastTrack Services

to verify the sample concentration, indicating the likelihood of success throughout the sequencing process. Short-insert libraries are prepared using TruSeq[®] technology from samples that meet the concentration requirements. Samples are batched using LIMS, and liquid handling robots perform library preparation to guarantee accuracy and enable scalability. All sample and reagent barcodes are verified and recorded in LIMS.

Quality Control

All DNA libraries undergo a two-step QC process before sequencing. This process includes qPCR to quantify DNA and gel electrophoresis to verify fragment sizes. QC results are evaluated in LIMS, and libraries that meet quality requirements are submitted for paired-end sequencing using HiSeq[®] Systems.

Sequencing

Following standard operating procedures, liquid handling robots denature and normalize libraries for cluster generation. Denatured libraries are then clustered using the cBot[™] System. After cluster generation, flow cells are submitted for sequencing on HiSeq Systems. Sequencing experts and engineers operate and maintain all HiSeq Systems to make sure that the best possible data are generated. A constant supply of clustered flow cells is available so that the systems are used at full capacity, enabling rapid turnaround times.

Quality Control

Throughout the sequencing process, experienced laboratory staff monitor the sequencing runs routinely. Cluster generation and sequencing are tracked through LIMS to verify proper run setup and sample tracking. Additionally, automated QC systems monitor sequencing run quality and aggregated information about the systems, reagents, and samples to ensure sustainability and optimal laboratory performance.

Data Analysis Process

Illumina scientists and project managers verify the quality of genomic data throughout the analysis process to make sure that the highest data quality is delivered. If a sample does not achieve the minimum coverage required, the project manager investigates the cause and notifies the researcher at the time of delivery. Data are delivered to the researcher in regular intervals until the project is completed, and project managers and scientists are available to answer any questions after delivery.

Researchers should check their data within two weeks after receipt to make sure that the data are complete. Illumina does not guarantee data storage after a project is complete. At the researcher's request, FastTrack Services will attempt to retrieve any data possible and will follow up on requests to replace data (at an additional cost).

Whole-Genome Sequencing Service

The FastTrack Services analysis pipeline contains several software components. Whole-genome sequencing data are processed through the Isaac Aligner and Variant Caller software¹ to detect single nucleo-tide polymorphisms (SNPs) and small insertions and deletions (indels). Other components of the analysis pipeline detect copy number varia-

tions (CNVs) and structural variants (SVs). Variants are delivered in industry-standard variant call format (VCF) and genome VCF (gVCF) for streamlined analysis.

In addition to the identified variants, aligned and unaligned sequence reads and quality scores are delivered to the researcher in archival BAM format for downstream analysis (Table 1). FastTrack Services use a reduced quality score resolution method, which decreases the BAM file size by 30%. The smaller BAM file reduces the time and cost associated with data storage and transfer without compromising accuracy. Sample summary reports, which include genome quality statistics and a Circos plot, are also delivered for every sample (Table 1). For more information about the analysis pipeline, refer to the Whole-Genome Sequencing Services User Guide².

Cancer Analysis Service

The Cancer Analysis Service is an add-on service to whole-genome sequencing, providing researchers with easy-to-interpret tumor/normal sequencing results. The analysis pipeline uses an optimized Bayesian combined calling method. In addition to the whole-genome sequencing data, the Cancer Analysis Service delivers somatic small-variant data and a somatic summary report to assess the quality of analysis (Table 1). For more information about the analysis pipeline, refer to the Cancer Analysis Services User Guide³.

Sample Verification by Genotyping

To verify positive sample tracking through the sequencing process, a second aliquot of every sample is processed through the Infinium[®] HD Assay using high-throughput Infinium BeadChips (Figure 2). The iScan System[®] scans the BeadChips and the variant data are analyzed using GenomeStudio[®] software. Illumina scientists use genotyping data to verify sequencing integrity by checking variant concordance^{*} between the two data sets. In addition to the sequencing data for each sample, FastTrack Services provide researchers with the genotyping data for variant confirmation (Table 1). The intensity files, product manifest file, cluster file, and sample sheet are also delivered so that the researcher can replicate the genotyping project as needed (Table 1).

Quality Control

The genotyping process is tracked through LIMS and is fully automated with liquid handling robots to ensure positive sample tracking. Control samples are included on every sample plate for real-time QC and data review. If a sample does not meet the concordance requirement, it is processed a second time to make sure that the concordance is accurate. Project managers will discuss any samples that fail subsequent investigation with researchers at the time of delivery.

Downstream Analysis

FastTrack Services include secondary analysis of sequencing data to detect SNPs, small indels, CNVs, and SVs. These services also deliver all sequence reads and quality scores for further analysis. All data sets are delivered in industry-standard formats to facilitate discovery during downstream analysis. Illumina has collaborated with select software partners⁴ to provide researchers with tertiary filtering and

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^{* 99.5%} variant concordance is expected for all non-cancer samples.

Deliverable	Whole-Genome Sequencing Service	Cancer Analysis Service [†]
Sequencing Summary Report		
Circos plot	\checkmark	
Base call quality score distribution	\checkmark	
Sequencing depth distribution	\checkmark	
SNP and indel assessment	\checkmark	
Variant and structural variant summary (SNPs, indels, CNVs, and SNVs)	\checkmark	
Indel length distribution	\checkmark	
Sequencing Data		
Aligned and unaligned reads and quality scores (BAM)	\checkmark	
Sequencing variants (VCF and gVCF)	\checkmark	
Small somatic variants (VCF)		\checkmark
Genotyping Data		
Variants (VCF)	\checkmark	
Intensity files (*.idat)	\checkmark	
Manifest file	\checkmark	
Cluster file	\checkmark	
Sample sheet	\checkmark	
Other Deliverables		
Informatics pipeline user guides	\checkmark	\checkmark
Somatic summary report		\checkmark
Cancer Analysis Service is available as an add-on service to the Who	le-Genome Sequencing Service	





annotation tools for visualizing and interpreting whole human genome sequencing data (Figure 3). These software partners offer multigenome comparison and cancer analysis packages to aid researchers in discovering the biological significance of their sequencing results.

Summary

FastTrack Services offer sample-to-data solutions that support a range of research applications. The collaborative environment, robust and automated processes, and highly accurate data contribute to the success of every FastTrack Services sequencing project, providing researchers with the confidence to take the next step in their genetic analysis studies.

Learn More

To learn more, visit www.illumina.com/services.

References

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- Illumina software partners, www.illumina.com/services/whole-genome-sequencing-services/ignsoftware-partners.ilmn

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