FastTrack Whole-Genome Sequencing Service Methodologies

Introduction

Illumina FastTrack Whole Human Genome Sequencing (WGS) Service is powered by the HiSeq® 2000 System, providing a high-quality genome at a minimum of 30× average coverage. Researchers can benefit from the service's efficiency and accuracy to advance their studies and quickly publish results.

In preparing scientific papers or presentations, researchers may need to cite the various methodologies used by the FastTrack WGS service. The following paragraphs briefly describe the general methodologies employed by FastTrack WGS to construct and sequence DNA libraries and generate WGS data. These descriptions include methodologies for TruSeq® DNA PCR-free library construction, TruSeq Nano DNA library construction, HiSeq v4 chemistry, and the Infinium® HumanOmni2.5-8 genotyping array.

gDNA Quantitation

Genomic DNA is quantified prior to library construction using PicoGreen (Quant-iT PicoGreen dsDNA Reagent, Invitrogen, Catalog #: P11496). Quants are read with Spectromax Gemini XPS (Molecular Devices).

Library Construction

Human WGS: PCR-Free Workflow
Paired-end libraries are robotically generated from 500 ng to1 µg of gDNA using the Illumina TruSeq DNA Sample Preparation Kit (Catalog #: FC-121-2001), based on the protocol in the *TruSeq DNA PCR-Free Library Preparation Guide.* Pre-fragmentation gDNA cleanup is performed using paramagnetic sample purification beads (Agencourt AMPure XP reagents, Beckman Coulter). Samples are fragmented and libraries are size-selected following fragmentation and end-repair using paramagnetic sample purification beads, targeting short fragment inserts. Final libraries are quality controlled for size using a gel electrophoretic separation system and are quantified.

Human WGS: Low-Input Workflow
Paired-end libraries are robotically generated from 100 ng of gDNA using the Illumina TruSeq Nano DNA Library Preparation Kit (Catalog #: FC-121-4003), based on the protocol in the *TruSeq Nano DNA Library Preparation Guide*. Pre-fragmentation gDNA cleanup is performed using paramagnetic sample purification beads (Agencourt AMPure XP reagents, Beckman Coulter). Samples are fragmented and libraries are size-selected following fragmentation and end-repair using paramagnetic sample purification beads, targeting short fragment inserts. Final libraries are quality controlled for size using a gel electrophoretic separation system and are quantified.

Clustering and Sequencing—HiSeq v4 Chemistry

Following library quantitation, DNA libraries are denatured, diluted, and clustered onto v4 flow cells using the Illumina cBot™ system. cBot runs are performed based on the *cBot User Guide*, using the reagents provided in Illumina HiSeq v4.

Clustered HiSeq v4 flow cells are loaded onto HiSeq instruments and sequenced at a minimum of 100 bp paired-end runs. Sequencing runs are performed based on the *HiSeq 2000 User Guide*, using Illumina HiSeq v4 Reagents. Illumina HiSeq Control Software (HCS) and Real-Time Analysis (RTA) used on HiSeq 2000 sequencing runs for real-time image analysis and base calling.

Genotyping

Samples are processed using Infinium chemistry, based on the *Infinium LCG Assay Guide*, and run on the HumanOmni2.5-8 array. Resulting intensity .idat files loaded into GenomeStudio® software (http://www.illumina.com/software/genomestudio_software.ilmn) to export genotyping calls.

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