Illumina FastTrack™ Services Whole-Genome Sequencing Methodologies

Introduction

Illumina FastTrack Services Human Whole-Genome Sequencing (WGS) Service is powered by the HiSeq X™ System, providing a high-quality genome at a minimum of 30× average coverage. Researchers benefit from FastTrack Services expertise, receiving efficient and accurate results that allow them to quickly publish results. In preparing scientific manuscripts or presentations, researchers may need to document methodologies used by FastTrack service. Below is a brief description of methodologies used by FastTrack Services to generate DNA libraries and obtain WGS data including: PCR-free library construction; sequencing by synthesis (SBS) chemistry; and genotyping arrays performed in parallel for sample concordance and gender evaluation on the Infinium® HumanOmni2.5-8 BeadChips.

DNA Quantitation

Genomic DNA is quantified before library construction using PicoGreen (Quant-iT™ PicoGreen® dsDNA Reagent, Thermo Fisher SCIENTIFIC, Catalog #P11496). Samples are measured using a SpectraMax Gemini XPS (Molecular Devices).

Library Construction—PCR-Free

Paired-end libraries are generated according to the *Illumina SeqLab DNA PCR-Free Library Prep Guide* protocol using automated liquid handlers. The libraries are prepared using 500 ng–1 µg input gDNA, with the Illumina DNA Sample Preparation HT Kit. Prefragmentation gDNA cleanup is performed using paramagnetic sample purification beads (Agencourt AMPure® XP reagents, Beckman Coulter). Fragmentation and end-repair are performed, followed by size-selection using paramagnetic sample purification beads to enrich for target insert sizes. Final libraries are quantified by qPCR and evaluated for quality using an Agilent Technologies 2100 Bioanalyzer with an Agilent High Sensitivity DNA Chip.

Clustering and Sequencing – HiSeq X

Following library quantitation, DNA libraries are denatured, diluted, and clustered onto patterned flow cells using the Illumina cBot™ System. cBot runs are performed following *cBot System Guide* using Illumina HiSeq X paired end cluster kit reagents.

Clustered patterned flow cells are loaded onto HiSeq X instruments and sequenced on 151 bp, paired-end, non-indexed runs. All samples are sequenced on independent lanes. Sequencing runs are performed based on the *HiSeq X System Guide*, using HiSeq X reagent kits. Illumina HiSeq Control Software, and Real-Time Analysis are used with the HiSeq X sequencers for real-time image analysis, and base calling.

Genotyping

All DNA samples submitted for WGS sequencing are genotyped in parallel on Infinium HumanOmni2.5-8 BeadChips following the *Infinium LCG Assay Guide*. The resulting raw intensity .idat files are loaded into GenomeStudio® Software to perform genotyping analysis and to assess gender concordance (http://www.illumina.com/software/genomestudio_software.ilmn).

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