IAB Genome

A reliable analytical tool widely used for its excellent coverage quality enabling deep insight into various genomes. Illumina TruSeq DNA PCR-free protocol offers a superior solution for DNA regions that are traditionally difficult to sequence, such as GC-rich regions, promoters, and repetitive content, together with a reduction of library bias and gaps.

Introduction

By clinical diagnostics, Whole Genome Sequencing (WGS) is widely used to analyse genome abnormalities such as small-scale or large-scale mutations. Considering the requirements of a market, IAB Genome has been chosen for its robustness, while undesirable errors occurring during PCR steps within library preparation are eliminated, and excellent coverage quality in data output is served.

Except clinical laboratories, the IAB genome is widely used in research studies, while increasing sequencing capacity implies the enhanced coverage and *de novo assembly* of the genome can be reached, no less in evolutionary studies, agricultural applications or drug development where is appreciated IAB´s workflow modification.

IAB Expertise

The IAB team's expertise in processing WGS is also related to the four-year collaborative project ENIGMA (Etalon of National Interpreted Genome Map), aiming to develop validated local digital genome standards of the Czech population. The normative IAB QC requirements are reflected in the DNA extraction procedure, adoption of strict quantitative/qualitative parameters to a DNA sample and DNA libraries, followed by the high level of standardisation of sequencing processes (iSeq, NovaSeq X Plus sequencing platforms) and ending up with an evaluation of data quality and integrity by application of bioinformatic analytical pipelines (DRAGEN, Illumina) as well as data storage.

Protocol

Sample

Human genomic DNA: input 1000 ng

Quality Control (DNA)

- DNA purity: A260/280 ~1,6-2,0
- DNA quantity: C > 60 ng/µl in total volume > 25 µl (min. 1500 ng gDNA)
- DNA integrity: Intact DNA with no degradation

Normalisation of the DNA samples

Mechanical fragmentation of the DNA sample

- Covaris M220 Focused-ultrasonicator
- Quality check of fragment length by capillary electrophoresis

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Library preparation

- TruSeq DNA PCR-Free protocol (Fig 1)

Library QC

- Quantification
- Quality check by capillary electrophoresis
- Normalization of libraries based on molarity

Pre-sequencing

- Optional low-yield pre-sequencing at iSeq 100 (Illumina, USA) (Fig 2)
- Target – to reach the optimal equimolarity of the libraries in the pool for high-yield sequencing at NovaSeq X Plus

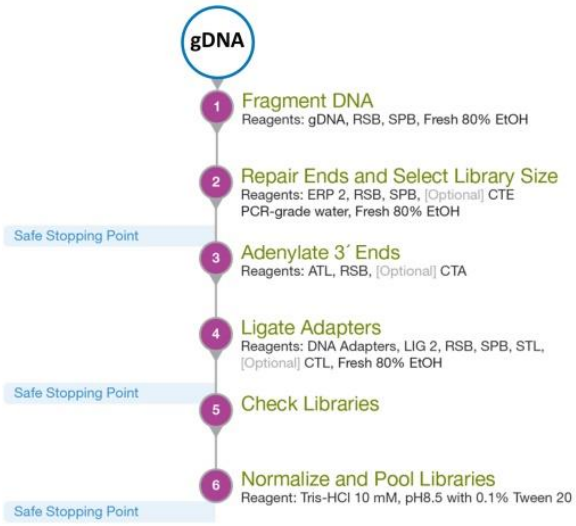

Figure 1

Sequencing

- NovaSeq X Plus (Illumina, USA)
- Pair-end sequencing 2×150 bp
- %Q30 > 75% (full NovaSeq X Plus specifications)
- Coverage target – Ø 30x/sample

Data analysis

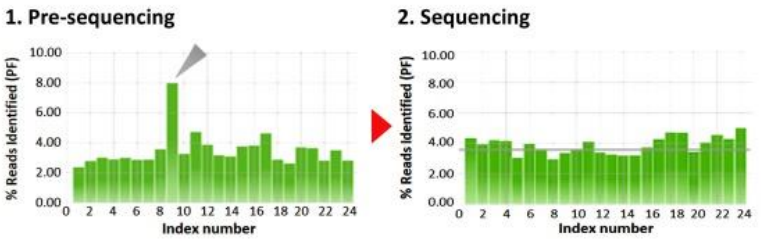

Figure 2

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Results

The bioinformatic team of IAB offers a complete solution for secondary and tertiary analysis of WGS data. Secondary analysis usually includes QC, trimming, reads mapping and variant calling (SNVs, InDels, CNVs, SVs). DRAGEN Bio-IT Platform (Illumina) provides accurate, comprehensive and efficient analysis for all these steps (Fig. 1, 2, 3). Emedgene (Illumina) covers the whole tertiary analysis for us, including variant annotation, prioritization and reporting.

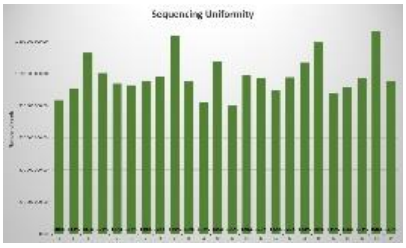

Figure 3

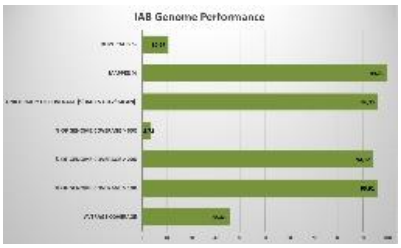

Figure 4

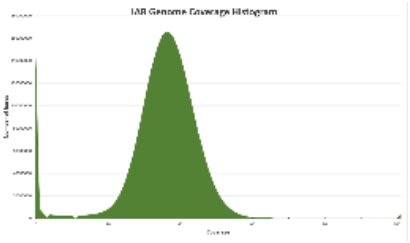

Figure 5

Conclusions

The IAB Genome provides a cost-effective solution for analysing chromosomal abnormalities, copy number variations (CNVs), gene fusions, germline variants, loss of heterozygosity (LOH), single nucleotide polymorphisms (SNPs), multiple-nucleotide variants (MNV), small insertions-deletions (indels), somatic variants, structural variants or de novo mutations in the human genome in combination with powerful Illumina sequencing technology.

About IAB

The experienced team follows IAB Genome workflow with strict quality procedures to perform the essential QC steps on your samples and generate reliable and repeatable results.

Illumina

Illumina is a global leader in sequencing and array technologies that are fueling groundbreaking advancements in life science research, translational and consumer genomics, and molecular diagnostics.

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