Whole Exome Sequencing Method

1. Experimental Procedure

1.1.Sample Quality Control

Please refer to QC report for methods of sample quality control.

1.2 Library Preparation, Sequencing

The genomic DNA was randomly sheared into short fragments with the size of 180-280 bp. The obtained fragments were end repaired, A-tailed, and further ligated with adapters. The fragments with adapters were PCR amplified, size selected, and purified. Hybridization capture of libraries was proceeded according to the following procedures. Briefly, the libraries were hybridized in the buffer with biotin-labeled probes, and magnetic beads with streptavidin were used to capture the exons of genes. Subsequently, non-hybridized fragments were washed out and probes were digested. The captured libraries were enriched by PCR amplification. The experimental procedures of DNA library preparation are shown in Figure 1.

The library was checked with Qubit and real-time PCR for quantification and a bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on DNBSEQ-T7 platforms with PE150 strategy, according to the effective library concentration and the data amount required.
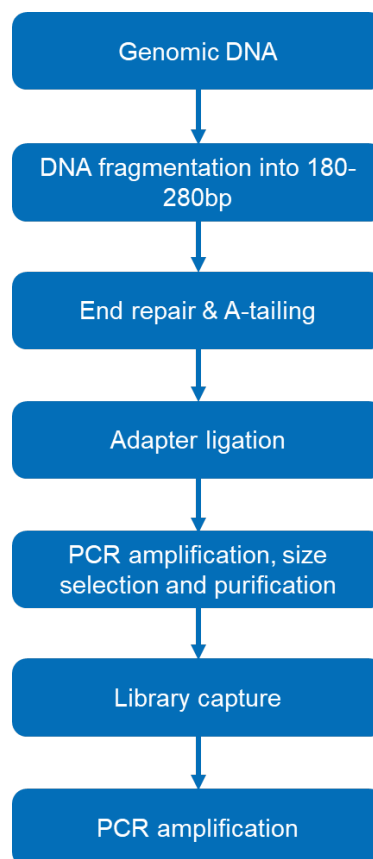

Figure 1. Workflow of Library construction

2. Bioinformatics Analysis

2.1 Raw data

The original fluorescence image files obtained from DNBSEQ-T7 platform were transformed to short reads (raw data) by base-calling and these short reads were recorded in the FASTQ format, which

contains sequences and the corresponding sequencing quality information.

2.2 Data Quality Control

It was the sequence artifacts, including reads containing adapter contamination, low-quality nucleotides, and unrecognizable nucleotide (N), that undoubtedly set the barrier for the subsequent reliable bioinformatics analysis. Therefore, quality control is an essential step to mitigate these obstacles and could be applied to guarantee meaningful downstream analysis.

The steps of data processing were as follows:

(1) Discard a pair of reads if either one of them contains adapter contamination (>10 nucleotides aligned to the adapter, allowing ≤ 10% mismatches);

(2) Discard a pair of reads if more than 10% of bases are uncertain (read as N) in either one of the reads;

(3) Discard a pair of reads if the proportion of low quality (Phred quality <5) bases is over 50% in either one of the reads.

Total reads number, raw data, error rate, and percentage of reads with Q30 (the percent of bases with Phred-scaled quality scores greater than 30) were calculated and summarized. After which, filtered reads were used as clean data for subsequent analysis.

2.3 Sequence Alignment

Clean data were mapped to the reference genome (b37/hg19/hg38) by Burrows-Wheeler Aligner (BWA) software (Li et al., 2018) to generate BAM files. Subsequently, Sambamba (Tarasov et al., 2015) was used to sort BAM files according to chromosome position. Picard tools (The Quick Start is available online: https://broadinstitute.github.io/picard/) were then utilized to merge BAM files and mark duplicate reads.

2.4 Variant Detection

2.4.1 Germline Mutation Detection

GATK (DePristo et al., 2011) HaplotypeCaller was used to call germline SNP and InDel, while the use of GATK VariantFiltration module was carried to filter germline SNP and InDel. The filter parameters of SNP and InDel are shown as follows:

SNP: QD < 2.0 || FS > 60.0 || MQ < 40.0 || HaplotypeScore > 13.0 || MappingQualityRankSum < -12.5 || ReadPosRankSum < -8.0

InDel: QD < 2.0 || FS > 200.0 || ReadPosRankSum < -20.0

2.4.2 Somatic Mutation Detection (Only for normal-tumor paired samples)

Somatic mutation detection is commonly applied to normal and tumor-paired samples. Somatic SNV was detected by MuTect (Cibulskis et al., 2013), while the somatic InDel was identified by Strelka (Saunders et al., 2012). Control-FREEC (Boeva et al., 2012) was used to detect somatic CNV. The parameter with window = 0 was set as officially recommended in the config file for somatic CNV detection.

2.5 Annotation

ANNOVAR (Wang et al., 2010) was used to perform variant annotation. Annotation contents refer to protein-coding changes, genomic regions affected by the variants, allele frequency, deleterious prediction, etc. The main databases used were as follows:

<u>Genes and regions annotation</u>

RefSeq (O'Leary et al., 2016) and Gencode (Frankish et al., 2021) databases were used to find genomic regions affected by variants and possible changes in the protein. We annotated the features of the genomic regions affected by the variants, such as cytoband, small RNA, conserved mammalian microRNA regulatory target sites, conservative regions of vertebrates, transcription factor binding sites, repeats, etc.

<u>Databases with frequency annotation</u>

The established databases, such as 1000 Genomes (1000 Genomes Project Consortium) (Abecasis et al., 2012), Exome Aggregation Consortium (ExAC) (Kobayashi et al., 2017), Genome Aggregation Database (gnomAD) (Pio et al., 2021) and exome sequencing project (ESP), were all used to find alternative allele frequencies in the populations that were reported. There are a great number of common polymorphism sites in the human population, while many deleterious variants are rare or of low frequency.

<u>Databases and scores with conservative and deleterious annotation</u>

SIFT (Ng et al., 2003), PolyPhen (Adzhubei et al., 2013), MutationAssessor (Reva et al., 2011), LRT (Chun et al., 2009), and CADD (Rentzsch et al., 2019) scores were used to predict the deleterious mutations. GERP++ (Huber et al., 2020) scores were used to evaluate the conservation of mutations. SIFT, Polyphen2, MutationTaster (Steinhaus et al., 2021), LRT, MutationAssessor, and FATHMM (Shihab et al., 2013) were all used to predict whether an amino acid substitution affected protein function. SiPhy (Garber et al., 2009), phyloP (Pollard et al., 2010), GERP++, and CADD were all used to predict the conservative level of the site. It should be noted that the conservation scores only consider the conservative level at the current site, but not the one involved in the nucleotide identity. Therefore, synonymous and non-synonymous variants at the same site will have the same scores. These scores are used for finding functionally important sites, which means that variants that confer increased susceptibility would score well.

<u>Databases with cancer and disease-related annotation</u>

dbSNP (Sherry et al., 2001), COSMIC (Tate et al., 2019), OMIM (Hamosh et al., 2005), GWAS Catalog (Buniello et al., 2019), and HGMD (Stenson et al., 2020) were used to find reported information of the variants.

<u>Databases with functional and pathway annotation</u>

Gene Ontology (Lee et al., 2004), KEGG (Kanehisa et al., 2000), Reactome (Jassal et al., 2020), and PID (Schaefer et al., 2009) databases were applied to provide functional or pathway annotation.

2. References

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