

## Human Whole Genome Sequencing

### 1. Sample Requirements

#### 1.1 Illumina platform (350 bp insert DNA Library)

Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™)
Genomic DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	OD260/280=1.8~2.0 no degradation, no contamination
Genomic DNA (PCR free)	≥ 1.5 µg	≥ 20 µL	≥ 20 ng/µL	
Genomic DNA from FFPE*	≥ 0.8 µg	-	-	Fragments should be longer than 1500 bp

\* FFPE: Formalin-fixed, paraffin-embedded

#### 1.2 PacBio platform (SMRTbell® DNA Library)

Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™)
**HMW Genomic DNA	≥ 10 µg (for Sequel I); ≥ 30 µg (for Sequel II)	≥ 50 µL	≥ 100 ng/µL	OD260/280=1.8-2.0; OD260/230=2.0-2.2; fragments should be ≥ 30 kb for Sequel I, ≥ 60 kb for Sequel II; no degradation, no contamination; no EDTA contained in DNA elution buffer.

#### 1.3 Nanopore platform (Ligation 1D DNA Library)

Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™)
**HMW Genomic DNA	≥ 10 µg	≥ 50 µL	≥ 40 ng/µL	OD260/280=1.8-2.0; OD260/230=2.0-2.2; fragments should be ≥ 30 kb; no degradation, no contamination; no EDTA contained in DNA elution buffer.

\*\* HMW: High Molecular Weight

### 2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 150 bp
Recommended sequencing depth	For tumor tissues: 50×, adjacent normal tissues and blood 30× For rare diseases: 30-50×
Data quality	Guaranteed ≥ 80% bases with Q30 or higher
***Turnaround time	4~5 weeks from verification of sample quality to data releasing without bioinformatic analysis

Platform	PacBio Sequel I/II
Read length	average > 10 kb for Sequel I average > 15 kb for Sequel II
Recommended sequencing depth	For genetic diseases: 10-20× For tumor tissues: ≥ 20×
***Turnaround time	7~8 weeks from verification of sample quality to data releasing without bioinformatic analysis

Platform	Nanopore PromethION
Read length	average > 17 Kb
Recommended sequencing depth	For genetic diseases: 10-20× For tumor tissues: ≥ 20×
***Turnaround time	6~7 weeks from verification of sample quality to data releasing without bioinformatic analysis

\*\*\*Turnaround time varies depending on the project volume.

### 3. Data Analysis Contents

Standard Analysis
Data quality control: filtering reads containing adapter or with low quality
Alignment to reference genome; statistics of sequencing depth and coverage
Variant (SNP, InDel, CNV, and SV) calling, annotation and statistics
Somatic variant detection (only apply for tumor-normal paired samples) SNP calling, annotation and statistics InDel calling, annotation and statistics CNV calling, annotation and statistics SV calling, annotation and statistics Display of Genomic Variants with Circos

Advanced analysis	Methods
Personalized analysis (Cancer & Disease)	HLA typing
	CRISPR/Cas9 Off-target Analysis
	Xenograft Tumor Analysis
	Integration Site Detection

Advanced analysis	Methods	
Cancer		Screening for Predisposing Genes (feasible if only normal samples are provided)
		Mutational Spectrum & Mutational Signature
	Driver gene analysis	Identification of Known Driver Genes
		Significantly Mutated Gene & Pathway Analysis
		Mutation Relation Test of Significantly Mutated Genes
		Identification of Driver Genes Based on Mutation Clustering Bias
		Identification of Driver Somatic CNVs
		Identification of Driver Mutations in Noncoding Regions
		Mutation Site Displaying
	Tumor heterogeneity analysis	Tumor Purity & Ploidy Estimation
		Intra-tumor Heterogeneity Analysis
		Tumor Evolution Analysis (One normal and at least 3 tumor samples from the same patient are needed)
		Fusion Gene Detection
		Tumor Neoantigen Identification

Advanced analysis	Methods	
Monogenic disease		Candidate Variant Filtration
		Analysis under dominant/recessive model
		Linkage Analysis
		Region of Homozygosity Analysis (ROH)
Polygenic disease		Candidate Variant Filtration
		Analysis under dominant/recessive model
		Linkage Analysis
		Region of Homozygosity Analysis (ROH)
		De novo SNV/INDEL Analysis

Advanced analysis	Methods	
Personalized analysis (Cancer & Disease)		HLA typing
		CRISPR/Cas9 Off-target Analysis
		Xenograft Tumor Analysis
		Integration Site Detection