

Animal & Plant *De novo* Sequencing

1. Sample Requirements

1.1 Illumina platform (insert \leq 500 bp DNA library)

Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Genomic DNA	\geq 200 ng	\geq 20 μ L	\geq 10 ng/ μ L	OD260/280 = 1.8~2.0; no degradation, no contamination
Genomic DNA (PCR-free non-350bp)	\geq 5 μ g	\geq 20 μ L	\geq 30 ng/ μ L	
Genomic DNA (PCR-free 350bp)	\geq 1.2 μ g	\geq 20 μ L	\geq 20 ng/ μ L	

1.2 PacBio platform (SMRTbell® DNA Library)

Library Type	Sample Type	Amount	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
PacBio sequel II DNA CLR library	* HMW Genomic DNA	\geq 5 μ g	\geq 50 μ L	\geq 70 ng/ μ L	Fragment size: most of DNA fragment is above 30K; OD260/280=1.75~2.0; OD260/230=1.5~2.6; **NC/QC=0.95~3.00
PacBio sequel II/IIe DNA HiFi library	HMW Genomic DNA	\geq 15 μ g	\geq 50 μ L	\geq 70 ng/ μ L	Fragment size: most of DNA fragment is above 30K; OD260/280=1.8~2.0; OD260/230=1.5~2.6; NC/QC=1.00~2.20

*HMW: High Molecular Weight

**NC/QC: NanoDrop concentration/Qubit concentration

1.3 Nanopore platform (Ligation 1D DNA Library)

Library Type	Sample Type	Amount	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Nanopore DNA library	*HMW Genomic DNA	\geq 8 μ g	\geq 50 μ L	\geq 100 ng/ μ L	Fragment size: most of DNA fragment is above 30K; OD260/280=1.75~2.0; OD260/230=1.4~2.6; NC/QC=0.95~3.00

*HMW: High Molecular Weight

2. Sequencing Parameters

GENOME SURVEY		
Platform	Illumina HiSeq Platform	
Sequencing Libraries	350 bp insert size	
Sequencing Strategy	PE150	
	SIMPLE GENOME <i>DE NOVO</i> SEQUENCING	COMPLEX GENOME <i>DE NOVO</i> SEQUENCING
Assembly Strategy I	50X PacBio Sequel long read data/Oxford PromethION reads data	
Data Quality Guarantee	Contig N50 \geq 1 Mb	Contig N50 \geq 300 kb
Assembly Strategy II	High quality de novo assembly (70X PacBio Sequel reads)	
Data Quality Guarantee	Contig N50 \geq 2 Mb	Contig N50 \geq 500 kb
Assembly Strategy III (Recommended)	Super-scaffold and chromosomal scale de novo assembly integrating PacBio Sequel reads/Oxford PromethION reads and Hi-C	
Data Quality Guarantee	Contig N50 \geq 2 Mb Scaffold N50 \geq 4 Mb	Contig N50 \geq 500 kb Scaffold N50 \geq 1 Mb

3. Data Analysis Contents

Standard Analysis (Assembly)
Data quality control: filtering reads containing adapter or with low quality
Genome assembly with long reads
Quality assessment of genome assembly

Standard Analysis (Annotation)
Repeat Sequence Annotation
Gene Structure Annotation
Gene Function Annotation
Non-coding RNA Annotation

Standard Analysis (Comparative genomic analysis)

Phylogenetic analysis

Gene family analysis

Positive selection analysis

Synteny

