

## Microbial *De novo* 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	≥ 10 µL	≥ 20 ng/µL	OD260/280=1.8~2.0; no degradation or contamination

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

Library Type	Sample Type	Amount	Volume	Concentration	Purity
PacBio Sequel II DNA CLR library	HMW Genomic DNA (Bacteria and Fungus)	≥ 2 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC=0.95~3.00 Fragments should be ≥ 20K
PacBio sequel II/IIe DNA HiFi library	HMW Genomic DNA (Fungus)	≥ 15 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.7~2.0; OD260/230=1.3~2.6; NC/QC=1.0~2.2 Fragments should be ≥ 20K

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

Library Type	Sample Type	Amount	Volume	Concentration	Purity
Nanopore DNA library	HMW Genomic DNA (Bacteria and Fungus)	≥ 6 µg	≥ 50 µL	≥ 60 ng/µL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC=0.95~3.00 Fragments should be ≥ 20K

### 2. Sequencing Parameters

Platform	Illumina Hiseq X
Read length	Paired-end 150 bp
Recommended sequencing depth	≥ 50x for bacterial and fungal genome
Data quality	Guaranteed ≥ 85% bases with Q30 or higher
*Turnaround time	Minimum 6 weeks from confirmation of library preparation to data releasing without bioinformatic analysis;

Platform	PacBio Sequel
Read length	≥ 10 kb or 20 kb
Recommended sequencing depth	≥ 100× for bacterial genomes ≥ 50× for fungal genomes
Data quality	Contig N50 ≥ 20 kb, Scaffold N50 ≥ 1 Mb
*Turnaround time	Minimum 6 weeks from confirmation of library preparation to data releasing without bioinformatic analysis;

\*Turnaround time varies depending on the project volume.

### 3. Data Analysis Contents

#### Bacterial and Fungal Draft Map Standard Analysis

Data quality control: filtering reads containing adapter or with low quality

Genome preliminarily assembles

Genome survey: genome size, GC distribution, repeat rate

Genome component analysis: gene structure prediction, repetitive sequences, non-coding RNAs

Gene function annotation: NR, GO, COG, KEGG, and Pfam

#### Bacteria Complete Map Standard Analysis

Data quality control: filtering reads containing adapter or with low quality

Genome advanced assemble

Genome survey: genome size, GC distribution, repeat rate

Genome component analysis: gene structure prediction, repeat sequences, non-coding RNAs, CRISPR, prophage analysis, gene island analysis

Gene function annotation: KEGG, SwissPort, NR, GO, COG

Genome cycle graph

#### Fungus Fine Map Standard Analysis

Data quality control: filtering reads containing adapter or with low quality

Genome advanced assemble

Genome survey: genome size, GC distribution, repeat rate

Genome component analysis: gene structure prediction, repeat sequences, non-coding RNAs

Gene function annotation: NR, GO, KOG, and KEGG