

## **Experimental procedures of Metagenomic sequencing**

### **1 Sample testing**

There are mainly two methods in QC for DNA samples:

1. DNA degradation degree and potential contamination was monitored on 1% agarose gels.
2. DNA concentration was measured using Qubit® dsDNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA).

OD value is between 1.8~2.0, DNA contents above 1ug are used to construct library.

### **2 Library construction**

A total amount of 1µg DNA per sample was used as input material for the DNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, the DNA sample was fragmented by sonication to a size of 350bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. At last, PCR products were purified (AMPure XP system) and libraries were analysed for size distribution by Agilent2100 Bioanalyzer and quantified using real-timePCR.

### **3 Sequencing**

The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina NovaSeq platform and paired-end reads were generated.