

## **Sample submission guideline**

### **Raw sample (for microbial DNA amplicon or genome sequencing)**

1. Raw material is required to keep in 2 ml clear safe-locked tubes such as Eppendorf tube, clear color (Cat. No. 022363352).
2. Each tube should be labeled clearly using permanent mark pen on either tube side or cap surface. We don't suggest using stick tags to label.
3. Sample tubes are sent to us in sample boxes. Any kind of boxes is available for us. The investigator's name, project, material, and date should be labeled on the front side of sample box. Lid of box should be sealed using tape in case of its opening during transportation.
4. Sample sheets are required, one copy sent to us with the samples and one copy through email.
5. Sample boxes should be shipped to us in a dry ice box.
6. Provider should let us know if antibiotic treatment is involved in the raw sample preparation for 16S rRNA sequencing.

### **DNA sample (for microbial amplicon or genome sequencing)**

1. Microbial DNA isolation: we suggest use the bead-beating based methods, such as PowerSoil DNA isolation kit (Mo Bio) or PureLink™ Microbiome DNA Purification Kit (Invitrogen), QIAamp DNA Microbiome Kit (Qiagen) etc. RNase A treatment and purification of DNA are needed.
2. DNA dilution: sample should be kept in 10mM Tris-HCl pH  $\geq$  8.0, or any dilution buffer in DNA isolation kits without EDTA. We don't suggest using H<sub>2</sub>O to dissolve DNA samples.
3. DNA quantification and quality check: several methods are available such as NanoDrop or Picogreen etc. The report of concentration (ng/ $\mu$ l) and quality (A260/A280, A260/A230) measurement is required when you send us DNA samples, which the ratio of A260/A280 for high quality DNA is  $\sim$ 1.8.
4. Sample normalization: all samples should be diluted to same concentration, 20 or 10 ng/ $\mu$ l for high concentration samples; 5 or 2 ng/ $\mu$ l for low concentration samples.

Although we usually need 20 ng sample for a sequencing run of 16S rRNA gene, some samples could be failed for some reason. Please send us 50 ng as minimum each so that we could repeat if it is necessary.

5. DNA sample store: sample should be stored in skirted 96-well PCR plates. The sample layout is as the order from column A to H. If samples submitted are more than 96, please leave the wells of G12 and H12 empty, which will be used for negative and positive controls. The investigator's name, project, DNA concentration and date should be labeled clearly on the side. For the sequencing of genome DNA such as metagenome sequencing, single tubes are acceptable.
6. Sample shipping: DNA plates or tubes should be shipped to us in a dry ice box or drop to our lab directly.
7. Sample sheet: Sample sheets are required, one copy sent to us with the samples and one through email. Sample sheet includes sample IDs, DNA original concentration and its quality report from NanoDrop, locations in 96-well PCR plate.
8. Provider should let us know if antibiotic treatment is involved in the raw sample preparation for 16S rRNA sequencing.

### **RNA sample (for RNA-seq, small RNA and Metatranscriptome sequencing)**

For RNA-seq and small RNA sequencing:

1. Only total RNA is accepted. Total RNA should be treated with RNase free DNase to remove DNA, and the treated RNA should be purified.
2. The amount of total RNA needed is between 1 to 5  $\mu\text{g}$ , and solved in RNase free water.
3. The methods of NanoDrop or Fluorometer such as Qubit are available for concentration measurement. Bioanalyzer system (Agilent) is suggested for RNA quality check, but not for concentration measurement. RNA quality report from Bioanalyzer is required when you submit the RNA samples.
7. Single tubes clearly labeled using permanent mark pen on either tube side or cap surface are accepted for RNA submission, and tubes should be kept in sample box. The information of investigator's name, project, material, and date should be labeled on the front side of sample box. Lid of box should be sealed using tape in case of its opening during transportation.
4. Sample boxes should be shipped to us in a dry ice box.

For metatranscriptome sequencing:

Contact us for a detail information of microbial RNA isolation and enrichment.

### **Sample naming**

1. Sequencer itself requires sample name ID for a running SampleSheet, and data analysis.  
To eliminate errors caused by typing sample IDs into the SampleSheet, we only copy and paste related information from the e-copy of your sample sheet.
2. Sample naming: includes three parts of [three characters for project], [three characters for experimental condition], [sample ID number], no space or “/” in the sample name, such as STEAviStlCtl002, WELGALN001, COLBRUS001.
3. We usually use PI’s last name, three characters, to represent the project.
4. Condition’s information is required when you want us to do bioinformatics analysis, for instance, GAL for gargle, BRU for brush, CTL for control.
5. Sample ID number: if total number is more than ten, use the first one is 01 instead of 1; if more than 100, the first one is 001 instead of 1.

### **Shipping address**

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