Metagenomics sample preparation guidelines

General requirements

IGATech offers nucleic acids extraction service and we can set up a dedicated extraction workflow for your specific substrate. Please enquire.

For sample number <12, send samples in Eppendof tubes sealed with parafilm (***0,5 mL and 0.2 mL tubes as well as strips will be not accepted***). The tubes must have, on the vial top, a clear and permanent sign (or a thin label) with a progressive number of the mailed samples and the customer's name (at least the initials).
For sample number above 12 it is mandatory to send samples in a skirted 96-wells plate associated with Sample spread sheet containing information on sample names and sample position. Send DNA samples in a cold pack (e.g. Blue ice) or dry ice. Do not ship plates without secondary containment as these may crack when placed directly on dry ice.

Please, do not forget to send us the compiled **Samples Spreadsheet**, both with the shipped parcel and via e-mail. In order to be able to properly track and safeguard your samples we also ask you to send us the **Tracking Number** via e-mail.

DNA for 16S - 18S - ITS amplicons

We suggest sending 200 ng of DNA at a minimum concentration of 10 ng/µL.
Mail DNA samples resuspended in water or 10mM Tris-HCl pH 8.5.
Sample acceptance is based on the amount and concentration measured by us at QBit fluorimeter. As we understand some substrates make hard to obtain high yields of DNA, we also accept sample below the recommended quantity. However, it's recommended, when possible, that customer run a test PCR (16S: 341F-805R; 18S: F655-R1200; ITS: ITS1-ITS4) to ensure that the sample are reaction-permissive as dirty substrates (soil, sludge, fecal, etc.) may have PCR-inhibitor leftover which can hamper amplification reaction.

Customer is free to add extra columns to Sample Spread Sheet to include all available metadata of samples. This information will be used to run several statistical tests as well as labeling/coloring of outputs in our standard internal analysis pipelines.

DNA for WGS metagenomics

Submit minimum 1 μg of DNA per sample. Please note that fluorimetry-based quantification (e.g. Qubit, plate-reader) assays are more accurate methods than absorbance-based methods (e.g. Nanodrop) which might overestimate the quantity.
Quality of the DNA should be 260/280 > 1.8 and 260/230 >1.8.
DNA must be resuspended in 10mM Tris-HCl pH 8.5 (standard elution buffer of most commercial column-based extraction kits); water is accepted as an alternative; no high concetration of EDTA must be present in the solution – e.g. TE buffer has 1mM EDTA - but consider 10mM Tris-HCl as best buffer for HMW DNA stability or 10mM Tris-HCl + 0.1 mM EDTA.

RNA for metatranscriptomics

Our workflow starts with minimum 5 μg of purified high quality total RNA per sample. During collection, it is important to immediately snap freeze the sample and store it in an RNA preservation buffer to protect RNA that is much less stable than DNA. In case total RNA was isolated from host-associated microbial communities, host mRNA is removed using poly(A) tail depletion, in such case, depending on the amount of host mRNA, the initial sample quantity must be raised up to hundreds of μg.

In order to obtain a high-quality sequencing data, customers must provide a good quality RNA, in detail:

- the 260/280 ratio of your RNA sample should be >1.8;
- samples should be resuspended in nuclease-free water;
- high-quality RNA should have two prominent bands (e.g. ribosomal RNA) on agarose gel; intensity of the 28S band (at 4.5 kb) should be twice of the 18S one (at 1.9 kb);
- RNA should have an RNA Integrity Number (RIN) > 8 on an Agilent Bioanalyzer 2100.
- RNA samples must be free of contaminating proteins and other cellular material, organic solvents (including phenol and ethanol) and salts.

Please note that use of degraded RNA can result in low yieldor failure of the protocol.