



## Human Microbiome 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 Eppendorf 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 compiled **Samples Spreasheet** and the **Customer registration form**, together with shipped samples as well as via e-mail.

### DNA for 16S human microbiome

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 but we cannot guarantee quality results.

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 has to 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 concentration 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 in order 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. We strongly recommend commercial kits for totalRNA or miRNA extraction (e.g. Spectrum Plant Total RNA Kit, TRI-REAGENT, RNeasy, MirVANA or MirPremier).

Please note that use of degraded RNA can result in low yield, over-representation of 3'ends of the RNA molecules or failure of the protocol.