Metagenomics sample preparation guidelines

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

DNA for 16S - 18S - ITS

We suggest sending 200 ng of DNA at a minimum concentration of 10 ng/µL, min volume 20µL. Send DNA samples re-suspended in water or 10mM Tris-HCl pH 8.5.
Sample acceptance is based on the amount and concentration measured by fluorimetry-based quantification (*e.g.* Qubit, plate-reader). Keep in mind that absorbance-based methods (*e.g.* Nanodrop) might largely **overestimate** the DNA quantity.

The A260:A280 and A260:A230 ratios for DNA samples should be > 1.8. Use of DNA with lower ratios may result in low amplification yield.

We understand that from some substrates it can be hard to obtain high yields of DNA. We also accept samples below the recommended quantity/purity. It is recommended, when possible, that customer runs a test PCR (16S: 341F-805R; 18S: F655-R1200; ITS:ITS1-ITS4) to ensure that the samples are reaction-permissive, as the DNA of "dirty" substrates (soil, sludge, fecal, *etc*.) may contain PCR-inhibitor leftover which can hamper amplification reaction.

See the **shipping information** below.

Amplicon DNA

In case you are sending amplicons, **specify in the Sample Spreadsheet whether Illumina overhang adapter sequences have been appended to locus-specific sequences** (primers):

Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[*locus-specific sequence*]
Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[*locus-specific sequence*]

More details can be found on page 3 of
https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

Make sure to **specify amplicon length** and **protocol used for purification**. In case you're sending a mix of amplicons specify also if their sequences are similar or highly variable.

We suggest sending purified amplicon in water or 10mM Tris-HCl pH 8.5 at a minimum concentration of 10 ng/µL, min volume 20µL. For amplicons longer than 500 bp send at least 500 ng in min volume 20µL.

See the **shipping information** below.

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.

The A260:A280 and A260:A230 ratios for DNA samples should be > 1.8. Use of DNA with lower ratios may result in low amplification yield.

DNA must be re-suspended 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 the best buffer for HMW DNA stability or 10mM Tris-HCl + 0.1 mM EDTA.

See the **shipping information** below.

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 will be removed using poly(A) tail depletion. Thus, depending on the amount of host mRNA, the initial sample quantity must be upraised up to hundreds of micrograms.

To obtain a high-quality sequencing data, customers must provide a good quality RNA:

- 260/280 ratio >1.8;

- 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.

Use of degraded RNA can result in low yield or failure of the protocol.

Shipping

For batches of <24 samples, send samples in 1.5 mL or 2 mL 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** corresponding to information specified in the **Sample Spreadsheet**.

For batches of >24 samples, send samples in a skirted 96-wells plate, sealed with adhesive/heat-sealed aluminum foil **ONCE YOU HAVE PERFORMED** a fluorimetry-based quantification and normalized the DNA quantities. DNA concentration across samples should be even within each plate (+/-20% of average). In case internal normalization service is waived, IGAtech is not responsible for the quality of the data in term of per-sample coverage.

Each plate must be labeled with a plate identifier and accompanied with the **Sample spreadsheet** containing information on sample names and sample position.

Ship samples in a cold pack (*e.g.* **Blue ice for DNA** and **dry ice for RNA**). Organize the packaging in a way to avoid damaging and dispersion during the shipment: place tubes and plates in smaller plastic bags or boxes. Put a separator between stacked plates to avoid perforations of adhesive foils and leakage.

Significant differences in nucleic acid concentration of samples can occur if samples are of different origin or extracted with different methods. This may cause different efficiencies during library construction. Please, organize plates by creating homogeneous groups and report all information in the **Sample Spreadsheet**.

It is **MANDATORY to send us the compiled Sample Spreadsheet**, both with the shipped parcel and via e-mail. To be able to properly track and safeguard your samples send us the Tracking Number via e-mail.

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

IMPORTANT: We remind you that human-derived samples must be anonymized. Therefore, we cannot accept samples that come along with personal identification data (name and surname, fiscal code, etc.). Supplementary data related to the study such as prognosis, biometrics values, age, sex and other information not directly associated to an individual can be provided with no limitation (please use the Sample Spreadsheet available in the Documents section).

If human samples are provided in the form of tissue or body fluid, please fill out the Human samples clearance form and return a signed copy.