Metabarcoding

Sample preparation and Shipping Guidelines

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SERVICE CONDITIONS

Please read carefully!

The samples must meet the quality and quantity criteria and the shipping conditions, as indicated in the "Sample Requirements" and "Shipping" sections below. In case such specifications are not available the Customer shall enquire before sending any material. If storing and shipping conditions or sample specifications do not meet the required standards, IGA Technology Services may ask for an additional processing fee and the standard turnaround time might be delayed. Please note that the experimental setup and processing will be based on the information reported in the **Metabarcoding Sample Spreadsheet (see Documents/Guidelines section in https://igatechnology.com/)**. In addition, for both DNA and amplicons, the checkpoint of the workflow will be performed on the final libraries. In the case of failure due to inconsistencies between values declared in the Sample Spreadsheet and observed measurements, IGA Technology Services will not be responsible for any lack of results or inadequate quality and quantity of sequencing data.

Different rules of engagement are applied depending on whether the Client has requested a "Value" or a "Focus" service level.

Metabarcoding - "Value" level

If some samples within a batch fail the amplification, while others succeed, the failure is deemed to be caused by sample properties, *i.e.* a lack of template DNA or PCR inhibitors, and IGA Technology Services is not obliged to repeat the experiment. The experiment will be repeated only when it is assessed that sample handling at IGATech caused amplification/sequencing failure by evidence of a specific pattern. Samples with low amplification yields can be loaded on the sequencer at the customer's request and full service will be charged, otherwise, only the library preparation will be charged for such samples.

Metabarcoding - "Focus" level

A "Focus" package allows one extra round of preparation for samples with too low amplification yields or poor sequencing yields. The second round of preparation will be carried out to recover them. Minor changes to the protocol might be applied to overcome the lack of amplification (*e.g.* dilution of input DNA or modification of annealing temperatures). After the second round of preparation, samples with low amplification yields can be loaded on the sequencer at the customer's request and full service will be charged, otherwise, only the library preparation will be charged for such samples.

Protocol Optimization

A protocol optimization workflow (for primers that have not been tested yet by IGATech or for which the Customer does not have a known working protocol) can be requested in front of an additional charge. The standard commitment will be to process a set of 8 reference samples (representative of customer's sample conditions) for which: two annealing temperature and three serial dilution of template DNA will be tested to verify an optimal setting. If the entire series of samples in a certain temperature/dilution setting shows successful amplification, and so the following test sequencing provides adequate results, the given amplification set-up will be validated as default for the coming

experiments from the Customer. If the best series provides less than 8 samples, the customer can decide to continue with such setting while accepting the risk of variable results and success rate in amplification and sequencing. In any case the rules of engagement for repetition of library preparation and sequencing will refer to the “value”/”focus” level chosen for the project.

Amplicon sequencing strategy. To ensure optimal sequencing quality, amplicons with an expected length of < 200 bp will be sequenced in paired-end 2 × 150 bp mode on either the NovaSeq X (Illumina) or AVITI (Element Biosciences) platform. Amplicons with an expected length of ≥ 200 bp will be sequenced in paired-end 2 × 300 bp mode on the AVITI (Element Biosciences) platform.

Extra processing fees

Extra processing fee can be applied in the following cases:

- Inclusion of PNA clamps in the reaction
- Custom oligo PCR setup
- Extra purification of DNA or PCR products
- In-process QC (input, purified PCR1, other)
- Input normalization.

Please enquire for more information.

Contacts

All requests for information about the service must only be sent via email (**metagenomics@igatechnology.com**) and will be dealt with within a maximum of two working days from receipt.
In case problems arise during the sample processing, the Customer will be contacted directly to agree on possible solutions. Notifications will be sent to the contact person indicated in the Sample Spreadsheet at the sample reception and delivery.

For sample return and data storage and delivery refer to general Terms and Conditions.

SAMPLE REQUIREMENTS

Pseudonymization.

Please refer to general Terms and Conditions.

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.

DNA extraction

IGA Tech offers nucleic acid extraction services from plants, soil and stool. We can set up a dedicated extraction workflow for your specific substrate. Please enquire.

DNA specifications

- Diluent: water or 10mM Tris-HCl pH 8.5
- Concentration: 5ng/uL – 50ng/uL (by fluorimetry)

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. The use of DNA with lower ratios may result in low amplification yield.

- Minimum volume: 25uL
- DNA concentration across samples should be even (+/-20% of average). In case internal normalization service is waived, IGAtech is not responsible for the quality of the data in terms of per-sample coverage.

Amplicon specifications (short-read sequencing)

- Diluent: water or 10mM Tris-HCl pH 8.5
- Concentration range: 5ng/uL – 50ng/uL (by fluorimetry)
- Minimum volume: 25uL

Obligatory! Indicate in the Sample sheet of the Sample Spreadsheet:

- Expected amplicon length
- Primer sequences used
- Illumina overhangs used:
    - Nextera

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

- TruSeq
  Forward overhang: 5’ CTCTTTCCCTACACGACGCTCTTCCGATCT-[locus-specific sequence]
  Reverse overhang: 5’ TGGAGTTCAGACGTGTGCTCTTCCGATCT -[locus-specific sequence]

- NO OVERHANG: **must be clearly stated during the quotation process.**

Library specification

In case the Customer is sending libraries or library pools for direct sequencing, please follow the Custom libraries Illumina or Custom libraries Element Bioscience sample preparation guidelines and shipping.

SHIPPING

For batches of <=24 samples, 1.5 mL or 2 mL tubes can be accepted. They must be sealed with parafilm. 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 Metabarcoding Sample Spreadsheet.
For batches of >24 samples, samples are only accepted in 96-well plate, sealed with adhesive/heat-sealed aluminum foil or multiwell strip caps. Each plate must be labeled with a plate identifier indicated on the Metabarcoding Sample Spreadsheet.

IMPORTANT!!! The 0,5 mL and 0.2 mL tubes as well as strips are not accepted !.

For more details, please follow "**Shipping and Packaging Guidelines**" PDF file available at

https://igatechnology.com/igatech/documents/

IF YOU HAVE ACTIVED THE PROJECT via https://customer-portal.igatechnology.com/:

Please follow the **shipping procedure** on the portal, attaching the **Sample Spreadsheet** and generating a shipping barcode to be insert into the parcel.

IF YOU RECEIVED THE QUOTATION via EMAIL

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

Shipping Address:
IGA Technology Services srl
c/o Parco Scientifico e Tecnologico "Luigi Danieli"
via Jacopo Linussio 51
33100 Udine Z.I.U.
Italy