Genotyping

Sample Preparation and Shipping Guidelines

Contents

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 Sample Spreadsheet **(see Documents/Guidelines section in https://igatechnology.com/).** 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.

Extra processing fees

Extra processing fee can be applied in the following cases:

- Extra purification of DNA
- Input normalization

Please enquire for more information.

Contacts

All requests for information about the service must only be sent via email (**genotyping@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 animal and plant tissues. We can set up a dedicated extraction workflow for your specific substrate. Please enquire.

DNA extraction and quantification suggestions:
We recommend using a column-based or a beads-based isolation system, which provides more reproducible DNA quality and quantity across samples. Alternative protocols such as CTAB methods are suitable as well. However, one should be aware that CTAB/phenol-chloroform extraction methods may leave contaminant residuals in the DNA, which can impair enzymatic reactions during library preparation. Moreover, CTAB leftover in the sample can cause over-estimation of DNA concentration when using absorbance-based instruments (Nanodrop). We therefore encourage to estimate DNA concentration with dsDNA-specific intercalating agents (e.g. Qubit, fluorometric plate reader).
For genotyping application which works on pooled samples, the DNA normalization is crucial, thus DNA must be provided at the same concentration for all samples (±20%). Failure in using normalized DNA will result in high sample-to-sample sequencing yield variation, hampering the quality of results. For this reason, we encourage our customers to provide accurate quantification of samples or to inquire for our internal quantification/normalization service.

DNA specifications

- Diluent: 10mM Tris-HCl pH 8.5

Do not provide DNA with EDTA; max concentration allowed < 0.1 mM. EDTA can interfere with the first reaction and result in assay failure or poor results.

- Concentration: 20ng/uL – 50ng/uL (measured by fluorimetry based methods)

Keep in mind that absorbance-based methods (*e.g.,* Nanodrop) might largely **overestimate** the DNA quantity. If DNA concentration has been measured using spectrophotometric methods, such as Nanodrop,

we strongly recommend customer supply us with twice the required amount (min. 50ng/µL and 1µg total). 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: 30uL

- 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.

- Fragments must be >20Kbp (an 0.8% agarose gel can be used on few samples to check for this requirement).

Batch specifications

If samples have different origins, such as tissue type, method of extraction, age of the specimen, it is expected to have significant differences in DNA concentration as well as different efficiencies in library preparation. IGATech will not take responsibility on variance caused by this factor and strongly encourage Customer to:

  - For large projects arrange the specimens that vary for quality/concentration on different plates (plates must be filled with 96 samples anyway).
  - Indicate different sample origins (along with quality and concentrations) in the Sample Spreadsheet.

Population information (ddRAD only)

- If the customer includes “population structure” information in the Sample Spreadsheet, it will be used to compare all populations pairwise and calculate summary metrics of diversity and inbreeding for each specified pair of populations. Otherwise, all samples in the set will be treated as a single group (metapopulation).
- If the samples come from genetic mapping crosses, the customer should provide “cross specifications” in the Sample Spreadsheet (such as cross type, parents, and progeny). Otherwise, the dataset will be treated as a metapopulation.
- If the customer wants to analyze ddRAD data using the reference pipeline, they should provide the genome reference and its link in the Sample Spreadsheet. Otherwise, the data will be analyzed using the de novo pipeline.

SHIPPING

Samples are only accepted in 96-well plates, sealed with adhesive/heat-sealed aluminum foil or multiwell strip caps. Each plate must be labeled with a plate identifier indicated on the Sample Spreadsheet.
Only for replacements batches of <8 samples, 1.5 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 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