Transcriptomics

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. In addition, 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.

Please enquire for more information.

Contacts
All requests for information about the service must only be sent via email (**transcriptomics@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.

RNA extraction

IGATech offers nucleic acid extraction services from plants and cell pellets. Please enquire.

General indications for all RNA submission

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

- Resuspension buffer: nuclease-free water or low-EDTA TE buffer;

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). Otherwise, if using an RNA isolation method based on organic solvents, we recommend column purification after isolation

- Purity: the 260/280 ratio of your RNA sample should be >1.8;

- Integrity: RNA Integrity Number (RIN) > 8;

Otherwise, on agarose gel a high-quality RNA should have two prominent bands (e.g. ribosomal RNA) and intensity of the 28S band (at 4.5 kb) should be twice of the 18S one (at 1.9 kb); If available, customers need to provide the result analysis of Agilent 2100 Bioanalyzer or, at least, gel-electrophoresis image showing the RNA quality

- Concentration: (see the specific application below) A fluorimeter value (e.g. QuBit)
  Keep in mind that absorbance-based methods (*e.g.* Nanodrop) might largely **overestimate** the RNA quantity

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

RNA for Stranded mRNA-Seq

- Minimum total amount: 60 ng;

  For de novo (paired-ends) application, we suggest sending a minimum of 200 ng.

- Minimum concentration of 0.6 ng/µL;
- Minimum volume of 20 µL.

RNA for Plant, Bacterial and Metatranscriptomic Total RNA-Seq

- Minimum total amount: 200 ng;
- Minimum concentration of 20 ng/µL;
- Minimum volume of 10 µL.

A **DNase I** step is mandatory after the RNA isolation. RNA that has DNA contamination will result in an underestimation of the amount of the RNA used and poor data quality.

For plant *de novo* application (paired-ends), we suggest sending a minimum of 2 µg.

RNA for Human/Mouse/Rat Total RNA-Seq

- Minimum total amount: 60 ng;
- Minimum concentration of 3 ng/µL;
- Minimum volume of 20 µL.

A **DNase I** step is mandatory after the RNA isolation. RNA that has DNA contamination will result in an underestimation of the amount of the RNA used and poor data quality.

- For **FFPE samples:**
    - Minimum total amount: 200 ng;
    - Minimum concentration of 10 ng/µl;
    - Minimum volume of 20 µL.

Total RNA protocol works with moderately degraded RNAs even if the success rate is not guaranteed. Please inquire.

RNA for Ultra-Low input sample

- Minimum total amount: 200 pg* or 500 cell pellets°;
- Minimum concentration: 10 pg/ul;
- Minimum volume: 20 ul.

*We can use (experimentally) quantities below 100 pg but the rate of duplicates will be above 50%, based on our tests. Please inquire about custom designs for the target depletion of unwanted transcripts (rRNA, abundant constitutive mRNA) with AnyDeplete technology.
For applications with lower input samples or degraded material, please inquire. We can discuss custom protocols and methods.

° Direct cell inputs should be optimized depending on cell type. Please inquire.

RNA for Virus detection by Amplicon-Seq

- Minimum volume: 50 ul.

QC will only be performed once the library step is completed.

RNA for smallRNA-Seq

- Minimum total amount: 200 ng* for total RNA from cells and tissues, 20 ng for isolated smallRNA;
- Minimum concentration: 2 ng/ul;
- Minimum volume: 10 uL.

For **exosome, serum and plasma** samples, the minimum amount requested is 15 µl of RNA eluate. For further information and details, please inquire.

RNA for full length cDNA Oxford Nanopore Technology

- Minimum total amount: 500 ng of total RNA;

- Minimum concentration: 25 ng/ul;
- Minimum volume: 20 uL.

RNA for Direct RNA Oxford Nanopore Technology

- Minimum total amount: 2ug of total RNA;
- Minimum concentration: 250 ng/ul;
- Minimum volume: 20 uL.

SAMPLES for RiboSeq (ribosome profiling on NGS)

- Frozen pelleted cells;
- Frozen tissues.

As it is not possible to provide a minimal sample size as a defined number of cells or weight of Tissue, please inquire.

Library specifications

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 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 Sample Spreadsheet.

Send RNA samples in dry-ice or lyophilized with GenTegraRNA or GENEWIZ RNA Stabilization Tubes.

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