RNA/small RNA sample preparation guidelines

RNA for RNA-Seq / smallRNA-Seq application

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

General indications for RNA submission

In order to obtain 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; the 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). Otherwise, if using an RNA isolation method based on organic solvents, we recommend column purification after isolation.

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.

Customers need to provide the result analysis of Agilent 2100 Bioanalyzer or, at least, gel-electrophoresis image showing the RNA quality. Quantify your RNA samples by spectrophotometer (e.g. Nanodrop) or fluorimeter (e.g. QuBit).
Sample acceptance is based on the amount, concentration and Bioanalyzer QC measured by us. Send RNA samples in 1.5-2ml Eppendorf tubes sealed with parafilm (**0.2 mL and 0.5mL tubes, as well as strip tubes, WILL BE NOT ACCEPTED**). If you have 24 samples or more, please put your samples in a 96-wells skirted plate (MANDATORY). Do not ship plates without secondary containment as these may crack when placed directly on dry ice.

The tubes must have, on the vial top, a clear and permanent sign (or a thin label) with the progressive number of the shipped samples in accordance with the LABEL_ID in the **Samples Spreadsheet**. Send RNA samples in dry-ice or lyophilized with RNAstable (Biomatrica).

Protocol-specific requirements

RNA for Stranded mRNA-Seq

The minimum total amount requested is 60 ng (minimum concentration of 0.6 ng/µL, a minimum volume of 20 µL). For *de novo* (paired-end) application, we suggest sending a minimum of 200 ng.

RNA for Plant Total RNA-Seq

The total amount requested is 1 µg (minimum concentration of 50 ng/µL, a 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 *de novo* application (paired-end), we suggest sending a minimum of 2 µg.

RNA for Bacterial Total RNA-Seq

The minimum amount of RNA requested is 200 ng (minimum concentration of 20 ng/µL, a minimum volume of 10 µL). A DNase I step is highly recommended after the RNA isolation. The presence of genomic DNA in the RNA sample may have adverse effects on downstream analytical platforms. Also, if the total RNA sample contains a significant amount of genomic DNA, it may be difficult to accurately quantify the RNA concentration.

RNA for Human/Mouse/Rat Total RNA-Seq

The minimum amount of RNA requested is 200 ng (minimum concentration of 10 ng/µL, a minimum volume of 20 µL). A DNase I step is highly recommended after the RNA isolation. The presence of genomic DNA in the RNA sample may have adverse effects on downstream analytical platforms. Also, if the total RNA sample contains a significant amount of genomic DNA, it may be difficult to accurately quantify the RNA concentration.
For **FFPE samples** we accept down to a total amount of 200 ng (minimum concentration of 10 ng/µl, a minimum volume of 20 µL). Total RNA protocol also works with degraded RNAs and FFPE RNAs even if the success rate is not guaranteed.

RNA-Seq for Ultra-Low input sample

The total amount requested is 200 pg in at least 20 uL low-EDTA TE buffer (recommended) or nuclease-free water. We can use (experimentally) quantities below 100 pg but the rate of duplicates is going to be above 50%, based on our tests. Please inquire for custom designs for the depletion of unwanted transcripts (rRNA, abundant constitutive mRNA) with InDA-C system.
For application with **lower input samples or degraded material,** please inquire. We can discuss custom protocols and methods.

RNA for TruSeq smallRNA-Seq

The total amount requested is 2 µg (minimum concentration of 200 ng/µl) of total RNA or 100 ng of previously isolated microRNA (minimum concentration of 10 ng/µl) in 10 µl of nuclease-free water or 10 mM Tris-HCl, pH 8.5. For lower input sample**,** please inquire.

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*Please, do not forget to send us the compiled **Samples Spreadsheet**, both with the shipped parcel and via e-mail. In order to be able to properly track and safeguard your samples we also ask you to send us the **Tracking Number** via e-mail.*
