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Génome Québec

SEPTEMBER 8, 2020

MASSIVELY PARALLEL SEQUENCING SERVICES

Centre d'expertise et de services Génome Québec

User Guide:
Illumina sequencing technologies – RNA-Seq

Version 7.0

Table of contents

Guidelines for RNA Samples

General Considerations

Please contact libprepservices@genomequebec.com for all technical questions.

When working with biological samples that are destined to be submitted for RNA profiling (e.g. RNA-Seq, small RNA-Seq), it is recommended to:

- wear new gloves and keep samples on ice.
- resuspend samples in commercial RNase-free water.
- avoid DEPC-treated water.
- avoid resuspending samples in buffers containing detergents (e.g. SDS) as these will inhibit the enzyme reaction used to synthesize cDNA. If Trizol is used to extract RNA samples, it is recommended to perform a final clean-up (e.g. with Qiagen mRNA clean-up kit) prior to submission.

Aliquots must be sent, not stock samples. All RNA samples must be submitted in 1.5 mL Eppendorf tubes.

Any amount of remaining sample material will be retained for 3 months after the requested service has been completed and will then be discarded.

Sample identification of the tubes must correspond exactly to what is specified in the Request Form.

RNA Sample Specifications

Total RNA is used as the starting material for all the protocols.

A volume of 2-4 µL is used to first assess RNA Quality Control.

Quality Control is performed on total RNA, even for samples for which small RNA profiling will be performed. This means that the Platform cannot account for the presence/absence of small transcripts in the sample. It is therefore not guaranteed that a library will be generated from a sample which has successfully passed the QC.

It is better to dilute concentrated samples (e.g. 1 µg/µL down to 100 ng/µL) and provide at least the minimal volume required rather than submitting samples with higher concentration but smaller volumes.

Table 1. Summary of sample requirements for the construction of libraries from RNA

Application	Amount (ng)	Minimal concentration (ng/µL)	Volume (µL)	RIN*
Eukaryotic gene expression (protein coding transcripts; mRNA)	500	30	15	>6.5
Prokaryotic gene expression	750	50	15	>6.5
Meta-transcriptomics (multiple species)	1000	63	25	>6.5
Coding and long non-coding transcripts profiling	500	63	12	>6.5
Small RNA profiling** (<200 nt)	1600	160	10	>6.5
QC only		5	5	

*RIN = RNA Integrity Number; assessed on a Bioanalyzer (Agilent). Lower RIN indicate that the reads will be limited to the 3’ region of the genes.

** The protocol takes advantage of the 5’-phosphate and 3’-hydroxyl group of mature miRNA. The presence/absence of other small transcripts cannot be guaranteed.

If replacement samples have to be sent, send the full amount required, no ‘top ups’ are accepted.

Additional fees will be applied for the QC of each replacement sample.

Service Request and Sample Submission

Please note that lab work will only start when all required documentations is provided.

Service Request Form

Login to your Nanuq account.

In the Request Section, click Add new Request and follow the instructions.

Do not use the Back button of your Browser to go back to previous pages. Use the menu on the left-hand side of the screen.

Incomplete Request Form are accessible in future sessions using the Request List option.

The Request Form will remain on a Draft status until the 'Submit' button is used. Draft Requests cannot not be processed, and delays should be expected. The Client Management Office can only approve submitted requests.

Sample Submission

In the Request Section, click Request List and choose the right one.

To submit new samples, go to the Sample Submission Section and click on "+ New DNA/RNA/Cell Samples".

Choose "RNA" in "Sample Category" drop down menu. If the samples are extracted at Genome Quebec, choose "yes" at the question "Do your samples need Extraction?" If your RNA is already extracted, choose "no".

Answer to the rest of the questions and fill in the sample submission table. Click on 'Submit'.

Do not add new samples or replacement samples to an existing file. In the same Request, start over with "+ New DNA/RNA/Cell Samples".

Do not use the Back button of your Browser to go back to previous pages.

Please note that since samples will be entered and processed in the same order as in the Sample Submission Sheet, it is strongly recommended to randomize samples according to their experimental condition to minimize the technical variability of the sequencing.

Due to the large numbers of samples that are processed at the Centre, we cannot guarantee that specific loading schemes can be honored; we reserve the right to sequence lanes over multiple runs as deemed appropriate and without prior notice. Specific schemes have to be entered in the Comments column of the Sample Submission Sheet.

Preparing Samples for Shipment

The shipment must include a printed waybill.

RNA samples should be sent on dry ice pellets (not large blocks).

For submissions of more than 24 samples, tubes must be put in an orderly fashion in boxes. Make sure to protect tubes if the parcel contains heavy objects such as dry ice blocks, to minimize risks of damaging tubes. For submissions of 24 samples or less, tubes could be in a Ziploc-type plastic bag.

The package must contain enough dry ice for those samples remain frozen until destination. Thawing during transportation could result in a loss of integrity.

Samples crossing the Canadian border should be shipped early in the week. All required documentation for customs must be duly included with the package. The use of clear phrases such as: "*Non-biohazardous biological sample*", "*Purified DNA from [species]*", "*For research use only*", and "*Of no commercial value*" will help expedite customs clearance.

Samples can directly be brought to the laboratory. However, visits must be coordinated with the platform personnel beforehand. Opening hours are between 8h00 to 12h00 and 13h00 to 16h00 from Monday to Thursday and 13h00 to 16h00 on Friday.

Only send aliquots from your samples. These will be kept 3 months after the completion of the service. They will be discarded unless the answer "Yes" has been selected for the question "Would you like to have your original samples/primers returned to you?" in the field "Original Sample Disposal" of the Request Form.

Shipping addresses

Please refer to the waybill for instructions on how to ship your samples.