MASSIVELY PARALLEL SEQUENCING SERVICES

Centre d'expertise et de services Génome Québec

User Guide: Illumina sequencing technologies – DNA-Seq

Version 7.0

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Guidelines for DNA Samples

General Considerations

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

The guidelines contained herein are aimed at providing you the best possible sequencing data within the quickest possible turnaround time. Any and all samples that do not conform to the guidelines expressed herein may be refused without compensation.

When submitting nucleic acids for sequencing using next-generation sequencing technologies, it is recommended to:

- submit samples of the highest possible quality and purity:
    - has an OD 260/280 ratio of 1.8 to 2.0
    - has an OD 260/230 ratio of at least 2.0
    - does not contain insoluble materials, RNA, chelating agents, divalent metal cations, denaturants, detergents or carry over contamination from the starting organism/tissue
- perform DNA extractions with commercial kits rather than homemade solutions,
- perform a cleanup step prior to submitting samples,
- resuspend DNA samples in 10 mM Tris-HCl pH 8.0 with 0.1 mM of EDTA (higher concentrations of EDTA will inhibit some enzymatic reactions),
- quantify samples using fluorometric-based methods rather than purely spectrophotometric-based methods (e.g. Nanodrop) which tend to overestimate sample concentrations, resulting in an inadequate amount of starting material,
- assess integrity on agarose gels,
- ensure that the amount and concentration of each sample be within the range specified in the DNA Sample specifications section,
- measure precisely and report the volume of each sample contained in each well using the accompanying Service Request Form. When volumes not correspond to the values in the Service Request Form, these will be taken into account.

The sample name of each sample must be correspond exactly as indicated in the Service Request Form.

DNA Sample Specifications

Should the sample volume be inferior to the minimum volume specified in the table below, it will either be diluted to an appropriate volume without prior client consent or will be outright refused.

A volume of 3 µL is used for DNA Quality Control (QC).

Maximal DNA concentration should not exceed 150 ng/µL.

Table 1. Summary of DNA sample requirements

Source of nucleic acid	Library Type	Required Quantity (ng)	Required Volume (µL)
	DNA Shotgun (with PCR)	150	25 to 50
	DNA Shotgun with gel extraction (with PCR)	500	25 to 50
	Shotgun PCRFree	1500	25 to 50
	WGBS	700	25 to 50
	Target Capture (SureSelect)	300	25 to 50
	Target Capture (Roche Nimblegen)	300	25 to 50
	Methyl Capture (Roche Nimblegen)	2000	25 to 50
ChIP DNA	ChIP-Seq[ϕ]	10	25 to 30

[ϕ] Fragment size range of ChIP DNA is between 100 and 300 bp.

If replacement samples are needed, please send the full amount required. Partially replacements ("top ups") will be refused.

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

A volume of 10-15 µL is required for Quality Control (QC) only projects.

Accepted Formats for DNA Samples

Samples must be submitted in 96-well plates regardless of the number of samples.

There should be one sample per library type per well.

Two types of 96-well plates are accepted:

- Eppendorf twin.tec, Full Skirt, Cat# 951020401 ← FIRST CHOICE
- Corning Thermowell GOLD, Full Skirt, Cat# 3752 ← SECOND CHOICE

We recommend the following sealing films:

- Life Technologies MicroAmp® Clear Adhesive Film, Cat# 4306311
- VWR Aluminum Foils for PCR and Cold Storage, Cat# 60941-074

Pipelines have been optimized for high throughput processing of samples. Introducing plates other than the two models specified above may lead to sample loss and damage to the robotic liquid handlers. Therefore, samples submitted in non-conforming plasticware will be re-plated in the proper plates. There will be additional fees for re-plating them.

If samples from multiple projects are submitted at once, they have to be on different plates.

The requirements mentioned above also apply for Quality Control only projects.

Service Request and Sample Submission

Note that lab work will only start after all required documentation 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 Form

Login to your Nanuq account.

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 "DNA" 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 DNA 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 that, for Methyl-Seq and ChiP-Seq projects, 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.

Samples plates must be sent on dry ice pellets. If the package contains heavy objects which could damage plates during transportation (e.g. ice dry blocks, ice packs), it is strongly recommended to protect the plate from impacts.

The package must contain enough dry ice to ensure that samples remain frozen up to destination. Thawing during transportation could result in a loss of adhesion of the seal. This could lead to sample loss or cross contamination between samples.

Samples crossing the Canadian border should be shipped at the beginning of the week to minimize the risks of having samples at the carrier's storehouse over the weekend in case of delays. 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 7H to 12H and 13H to 16H from Monday to Thursday and 13H to 16H on Friday.

Only send aliquots from your samples. These will be kept 3 month 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.