# PacBio Sequel IIe 8M ZMW Sequencing Services

This document provides general information on the PacBio sequencing services offered by the Advanced Genomics Technologies (AGT) Laboratory under the direction of Professor Ioannis Ragoussis (ioannis.ragoussis@mcgill.ca) at the McGill Genome Centre.

Using the PacBio Sequel IIe Platform, we offer

- High molecular weight genomic DNA extraction
- Amplicon sequencing
- Full length RNA sequencing (cDNA)

We understand that different projects have specific needs. For any questions regarding your nucleic acid samples please contact pm.genome@mcgill.ca. If you would like to submit tissue/blood samples or material for nucleic acid extraction, please contact us in advance to discuss how to proceed.

## Make a service request

First create a user account on our website (hercules.genome.mcgill.ca) and follow the instructions for the service request. Our team of project managers will evaluate the pricing and feasibility of your project and will contact you to proceed with the project.

## Preparation of samples

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

### DNA Sample recommendations

For human, animal, and plant genomes, 90% or more of the DNA should be ≥ 10 kb, and 50% or more ≥ 30 kb, as measured on the Femto Pulse system. That corresponds to a genome quality number (GQN) of 9.0 or higher with 10 kb cutoff and 5.0 or higher with 30 kb cutoff.
Size requirements are less stringent for microbial and metagenomic samples. The DNA should be at least as large as the recommended insert lengths of 7–12 kb. Any degradation should be due to shearing from the extraction process (e.g., bead beating) and not from poor sample handling or storage, or biochemical processes.

- submit samples of the highest possible quality and purity. Good quality 260/280 ratio (ideally 1.8) and 260/230 ratio (ideally 2.0-2.2)
- DNA of high molecular weight is ideal for genomic DNA (peak at ≥45 kb)

- for HMW DNA, a concentration of 30 ng/ul or higher is preferred, especially if size selection is required
- DNA should be eluted in neutral, buffered solution (e.g., 10 mM Tris, pH 7.0 – pH 8.0). Do not use nuclease-free water or unbuffered solutions as this is insufficient for long-term DNA stabilization. Avoid buffers containing EDTA to prevent enzymatic inhibition during downstream sample library preparation
- avoid resuspending samples in buffers containing detergents (e.g. SDS) or other additives that can inhibit enzymatic reactions during the library preparation and/or sequencing reaction
- If submitting PCR products: they should be clean amplicons, without non-specific products or multiple bands. Perform a post-PCR cleanup step before submitting the samples.
    - Please provide us with the gel images where applicable
- quantify samples using the PicoGreen or Qubit method. Nanodrop or other purely spectrophotometric-based methods tend to overestimate sample concentration, resulting in an inadequate amount of starting material
- ensure that the volume of each sample provided should be within the sample specifications section described below
- precisely measure and report the volume of sample contained in each well in the sample manifest form. Sample identification of the container(s) carrying the sample(s) must correspond exactly to what is specified in the form
- please aliquot your samples in 10-75 ul
- if you have >8 samples for submission, provide them in 96-well fully skirted Eppendorf plates[2]

## RNA Sample recommendations

- The 260/280 optical density ratio must be ~2.0, while the 260/230 ratio must be equal to or greater than 2.0.
- Use RNase-free water, do not use diethylpyrocarbonate treated water (DEPC-treated).
- If Trizol is used to extract RNA samples, it is recommended to perform a final cleaning (ex. with the Qiagen mRNA cleaning kit) before submission.
- Avoid vortexing or pipetting quickly when working with RNA.
- Do not expose RNA samples to fluorescent intercalating dyes or ultraviolet radiation.
- RNA storage: use RNALater to stabilize and protect cellular RNA
- Always store RNA at -80°C, regardless of storage time.

Below you can find the required volume and concentration of nucleic acid samples and the suggested containers for sample submission:

| Sample type | plexing | conc/sample | volume needed | RNA integrity number | Recommended container |
|---|---|---|---|---|---|
| HMW DNA genome >=1Gb | none | 1 ug per Gb | | | 1.5 mL Tube[1] |
| HMW DNA | Up to 96 | 300 ng | | | 1.5 mL Tube[1] |

| | | | | | |
|---|---|---|---|---|---|
| genome <500Mb | | (total mass ≥1 µg per SMRTcell) | | | |
| RNA | Up to 96 | 300ng | | RIN >= 7 | 1.5 mL Tube[1] |
| Tissue | N/A | 25 mg | | | 1.5 mL Tube[1] |
| HMW if used gel selection | none | 1.5 ug per Gb | | | |

[1]1.5 mL LoBind Tube - Eppendorf catalog #22431021
[2] 96 well PCR LoBind plate – Eppendorf catalog #30129512

The tubes must be clearly identified with sample name. If several tubes are submitted at the same time, please ensure that each of them is labeled legibly. The names on the tubes must match those on the manifest.

**Sending/delivering the samples**

It is preferable that nucleic acid samples are delivered frozen or on ice. Samples can be brought directly to the McGill Genome Centre, such shipments must be coordinated with authorized personnel (listed below) prior to delivery.

Cryopreserved samples must be transported either on dry ice in an appropriate container (Styrofoam insulating box) or in a cryogenic cylinder for transport in liquid nitrogen. If shipping, make sure the package bears the Class 9 mark and the total weight of dry ice contained in the cooler according to the regulations for the transportation of dangerous goods in Canada.

The shipment should be organized so that reception takes place during working days to ensure that the integrity of the sample is preserved.

Samples should be sent to the following address:

***McGill Genome Centre***
***740 Avenue du Dr. Penfield, Montréal (Québec), H3A 0G1***
***AGT Nanopore Dept. Lab 5300***
***Tel: (514) 398-3311 ext. 00318***

Transport costs are the responsibility of the customer and the tracking number provided by the transport company must be communicated to us by email at the following addresses:

haig.djambazian@mcgill.ca
pierre.berube@mcgill.ca

Confirmation of receipt will be sent to you mentioning the condition of the package on arrival.

**Document to be attached to samples**

Your shipment must be accompanied by the following sample manifest document (available at https://www.mcgillgenomecentre.ca/hercules-resources/), filled with all the information related to your samples. You can contact us if you need to provide more specifications than the model allows. (See contact persons below)

This completed document must be returned to us by email at the same addresses as for the tracking number and a hard copy must be included in your package.

A valid purchase order (PO) corresponding to the statement of work (SOW) must also be received prior to starting the processing of your samples.

**After sequencing**

Following sequencing and fastq generation and raw data release a second level of quality control is performed to verify that the nanopore sequencing metrics are within acceptable range. The resulting expression matrices will be made available. Alignment bam files will be made available upon request.

**For more information**

For more questions, please contact us by email: pm.genome@mcgill.ca

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