



## Nanopore Sequencing Services

This document provides general information on the following single cell genomic services offered by the Advanced Genomics Technologies (AGT) Laboratory under the direction of Professor Ioannis Ragoussis ([ioannis.ragoussis@mcgill.ca](mailto:ioannis.ragoussis@mcgill.ca)) at the McGill Genome Centre.

Using Nanopore Flongle, MinION, or PromethION Platform, we offer

- High molecular weight genomic DNA sequencing
- Amplicon sequencing
- Direct RNA sequencing
- And more upon request

We understand that different projects have specific needs. For any questions regarding your nucleic acid samples please contact [pm.genome@mcgill.ca](mailto: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](http://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:

- 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  $\geq 45$  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<sup>2</sup>

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	Recommended container
fragmented DNA	20-96	1 pmol (or 200 ng)	preferred >10 ng/ul (min. 5 ng/ul)	96-well Plate <sup>2</sup>
fragmented DNA	2-20	2 pmol (or 400 ng)	preferred >10 ng/ul (min. 5 ng/ul)	96-well Plate <sup>2</sup>
HMW DNA	none	1.5 ug		1.5 mL Tube <sup>1</sup>
HMW DNA	none	500 ng		1.5 mL Tube <sup>1</sup>
fragmented DNA	none	200 fmol		1.5 mL Tube <sup>1</sup>
Bacteria WGS	up to 23	200 ng		96-well Plate <sup>2</sup>
RNA extract for Covid sequencing	up to 384		min. 15 ul RNA extract	96-well Plate <sup>2</sup>

<sup>1</sup>1.5 mL LoBind Tube - Eppendorf catalog #22431021

<sup>2</sup>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 5500**  
**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:

[sarah.reiling@mcgill.ca](mailto:sarah.reiling@mcgill.ca)  
[shu-huang.chen@mcgill.ca](mailto:shu-huang.chen@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](mailto:pm.genome@mcgill.ca)

#### **Laboratory Contacts:**

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