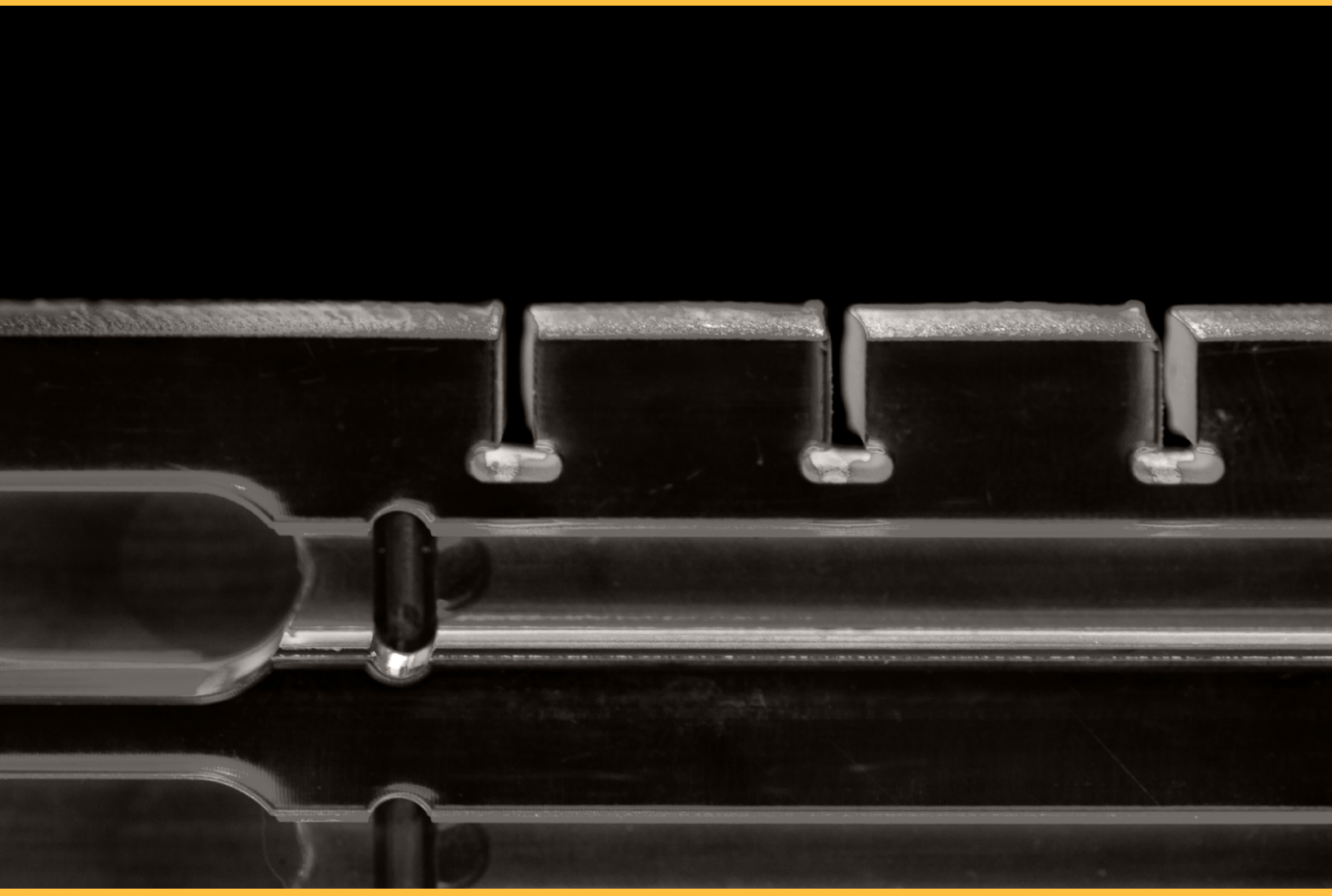
CANADA'S MICHAEL SMITH
GENOME SCIENCES CENTRE

From Nobel roots.

DNA



USER GUIDE

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Canada's Michael Smith Genome Sciences Centre
100–570 West 7th Avenue · Vancouver BC V5Z 1B3 · Canada
bcgsc.ca · *info@bcgsc.ca*

ABOUT US

Our **CAP**, **DAP, ISO 15189,** and **ISO 27001** certified technology platform is a high-throughput, large-scale DNA and RNA sequencing and analysis facility that has been designed to maximize analytical capacity, diversity, efficiency, scalability and flexibility.

Our state-of-the-art clinical, sequencing, bioinformatics, and proteomics platforms are ready to be put to use for your research or clinic.

We partner with researchers, guide experimental design, execute high quality processing of complex and valuable biological samples and provide extensive bioinformatics analyses with the aim of making genomics research accessible to our partners and collaborators within the scientific community.

Please feel free to contact us at *SOW@bcgsc.ca* if you have any questions about the services we provide.

ABOUT THIS GUIDE

This user guide describes requirements and recommendations for the preparation and submission of **extracted DNA**.

The recommended starting materials and amounts described in this guide work well for human or mouse derived nucleic acids. *For best results, please submit the recommended amount of starting material or more.*

If working with other organisms, please file a **Collaborative Services Inquiry** at *https://bcgsc.ca/services/collaborative-services-inquiries*.

If you are submitting a different sample type, please refer to the other user guides at *bcgsc.ca/services*.

CONTACTS

Submissions
GSC_submissions@bcgsc.ca

Statements of Work
SOW@bcgsc.ca

Data Support and Access
data_support@bcgsc.ca

Shipping
GSC_submissions@bcgsc.ca

General
info@bcgsc.ca

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1. SAMPLE PREPARATION

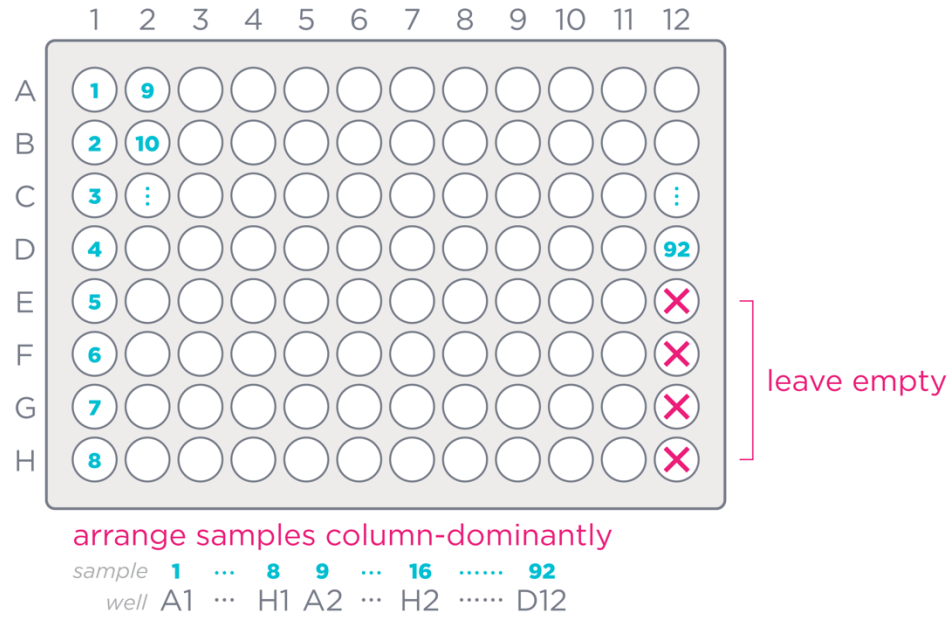
1.1. SUBMITTING EXTRACTED DNA FOR LIBRARY CONSTRUCTION

DNA samples are accepted in the following formats:

1. *Less than 24 samples:* **1.5 mL snap-top Eppendorf tubes** (or equivalent). Screw cap tubes are not accepted.
2. *24 or more samples:* **Axygen 96 FS-C plates**. If these are not available in your lab, you will be able to request a plate to be sent to you through our online submission system.

    2.1. In special circumstances, *24 or more samples* may be submitted in **tubes**. Please contact *GSC_Submissions@bcgsc.ca* for approval.

If submitting in a 96-well plate format, arrange samples in a column-dominant format with no empty wells between samples (e.g., A1, B1, C1, … H1, A2, B2, …) (see figure below). Wells E12, F12, G12 and H12 **must be left empty** for internal controls.



2. LIBRARY PROTOCOLS

2.1. TERMS USED

DNA	DNA (of any type, e.g., cfDNA or gDNA)
cfDNA	cell-free DNA
gDNA	genomic DNA (gDNA)
TNA	total nucleic acid
HMW DNA	high molecular weight DNA
A260/280	spectrophotometry ratio, used for quality assessment
A260/230	spectrophotometry ratio, used for quality assessment

2.2. SUBMISSION REQUIREMENTS

starting material	amount			quantification	quality assessment
	recommended	min	concentration		
PCR-FREE GENOME					
gDNA/TNA	**1,000 ng**	**700 ng**	**> 17.5 ng/µL** in **25–40 µL**	¶	†
FFPE GENOME					
gDNA/TNA	**1,000 ng**	**500 ng**	**> 12.5 ng/µL** in **25–40 µL**	¶	†
EXOME OR SPECIFIC CAPTURE (FFPE)					
gDNA/TNA	**1,000 ng**	**500 ng**	**> 12.5 ng/µL** in **25–40 µL**	¶	†
AMPLIFIED GENOME					
gDNA/TNA	**300 ng**	**25 ng**	**> 0.625 ng/µL** in **25–40 µL**	¶	†
EXOME OR SPECIFIC CAPTURE (AMPLIFIED GENOME)					
gDNA/TNA	**300 ng**	**25 ng**	**> 0.625 ng/µL** in **25–40 µL**	¶	†
GENOME TAGMENTATION					
gDNA/TNA	**120 ng**	**30 ng**	**> 1.0 ng/µL** in **20–30 µL**	¶	†
EXOME AND SPECIFIC CAPTURE (TAGMENTATION)					
gDNA/TNA	**120 ng**	**30 ng**	**> 1.0 ng/µL** in **20–30 µL**	¶	†
ChIP					
gDNA	**10 ng**	**5 ng**	**> 143 pg/µL** in **20–35 µL**	¶	‡
CIRCULATING CELL-FREE GENOME					
cfDNA	**25 ng**	**10 ng**	**> 0.25 ng/µL** in **40 µL**	–	–

¶ Quant-iT or Qubit dsDNA HS assay

† Spectrophotometry A260/280 1.8–2.0 and A260/230 2.0–2.2

‡ Agilent Bioanalyzer PAGE (if possible)

3. SUBMITTING FOR SPECIFIC PLATFORMS

3.1. NANOPORE

Nanopore sequencing can be performed on a variety of gDNA types, from short fragments (e.g., amplicons) to high molecular weight gDNA (HMW gDNA). Each sample can be run on its own flow cell (singleplex), or multiple samples can be indexed and pooled and run on one flow cell (multiplex). We use Femto Pulse (Agilent) QC to evaluate DNA fragment sizes and perform Blue Pippin (Sage Bioscience) or Short-Read Eliminator kit (PacBio) size-selection as needed.

The following requirements apply to all sample submissions destined for sequencing on the Nanopore platform:

1. DNA must be dissolved in 10 mM Tris at pH 8.0–8.5 (e.g., Qiagen EB Buffer)
2. Quantification method: Qubit assay
3. Quality assessment method: Spectrophotometry
4. *Quality value:* A260/280 1.8–2.0, A260/230 2.0–2.2.

3.1.1. ADAPTIVE SAMPLING

If adaptive sampling is requested, a BED and/or a FASTA file of targets will be required. A specific reference sequence for alignment also needs to be specified at submission. For further information contact *GSC_Submissions@bcgsc.ca*.

3.1.2. HMW gDNA FROM FROZEN TISSUES, CELLS, OR BODILY FLUIDS

For sequencing of HMW gDNA extracted from frozen tissues, cells, or bodily fluids, we strongly recommend size-selection to deplete DNA molecules < 10 kb in length.

NANOPORE PLATFORM				
	SINGLEPLEX or MULTIPLEX		MULTIPLEX	
starting material	up to 4 samples/pool		> 4 samples/pool	
	amount		amount	
	rec'd	min	rec'd	min
HMW gDNA or gDNA	**5 µg** **> 167 ng/µL** in **30 µL**	**2 µg** **> 22 ng/µL** in **47.5 µL**	**2 µg** **> 67 ng/µL** in **30 µL**	**400 ng** **> 9 ng/µL** in **47.5 µL**

3.1.3. SHORT FRAGMENTS

For sequencing of short fragments, submission amounts are variable, and depend on both the size of the fragments and level of multiplexing.

NANOPORE PLATFORM		
starting material	SINGLEPLEX or MULTIPLEX	MULTIPLEX
	up to 4 samples/pool	> 4 samples/pool
	amount	amount
	rec'd	rec'd
very small fragment DNA (50–200 bp)	**65 ng** **> 1.35 ng/µL**	**15 ng** **> 0.3 ng/µL**
small fragment DNA (200–3,000 bp)	**100 ng** **> 2 ng/µL**	**35 ng** **> 0.75 ng/µL**

3.1.4. OTHER SCENARIOS

For samples where recommended amounts are not available, size-selection can be bypassed if the submitter accepts the risk of shorter N50 read lengths and sequencing yields from the PromethION.

3.2. PACBIO REVIO

High Molecular Weight (HMW) gDNA is critical to ensure high quality long read data.

Because SMRT PCR-free genome sequencing doesn't involve PCR, the required input amount is relatively high as well.

PACBIO REVIO PLATFORM								
starting material	SINGLEPLEX				MULTIPLEX			
	1 sample, 1 SMRT cell				multiple samples, 1 SMRT cell			
	amount				amount			
	rec'd	min	max	conc.	rec'd	min	max	conc.
HMW gDNA or gDNA	**> 6 µg**	**0.5 µg**	**100 µg**	**> 15 ng/µL** in **30–300 µL**[1]	**> 6 µg**[2]	**0.5 µg**[3]	**100 µg**	**> 5 ng/µL** in **30–300 µL**[1]

[1] low TE or EB buffer

[2] in total for a pool of samples

[3] per sample (combined quantity per SMRT cell must be > 5 µg)

HMW gDNA is best eluted in Qiagen Elution Buffer (EB) or similar buffered solution (10 mM Tris at pH 8.0–8.5). Gentle handling at each step using wide bore tips for pipetting, and avoiding freeze & thaw cycles is recommended.

Ideally, buffers should not contain any chelating agents (e.g., EDTA), divalent metal cations (e.g., Mg^{2+}), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., SDS, Triton-X100).

HMW gDNA should not contain carryover contamination from the starting organism/tissue (e.g., heme, humic acid, polyphenols, polysaccharides, etc.).

Use low bind microcentrifuge tubes wherever possible.

Optimal samples will have OD260/OD280 1.8–2.0 and OD260/OD230 2.0–2.2.

Please share with us any sizing QC information about your sample, such as what would be obtained from Agilent Bioanalyzer, Tapestation, Femto Pulse, standard or pulse field gel electrophoresis.

3.3. STORAGE AND SHIPPING OF HMW gDNA SAMPLES

HMW gDNA samples are best stored and shipped at 4°C. Please ship with ice packs or wet-ice (not frozen). Samples can also be shipped at room temperature as an alternative.

Freezing is not recommended because freeze-thaw cycles shorten the DNA.

However, if your HMW gDNA samples are already frozen, they should be shipped with dry ice or frozen ice packs.

For PCR amplicons and samples in 96-well plates, frozen shipments are preferred.

Please refer to the Sample Shipping section for more details.

4. ONLINE SAMPLE SUBMISSION

Refer to **Introduction to Our Collaborative Services guide** for instructions on using the **Online Sample Submission System**. We recommend that you fully review this guide to working with us before initiating your submission.

The online sample submission form must be completed and approved prior to submitting your samples to the GSC.

If you have any questions, contact us at *GSC_Submissions@bcgsc.ca*.

5. SAMPLE SHIPPING AND DROP-OFF

Before shipping your sample, please make sure that your sample submission form has been reviewed and approved.

5.1. STORAGE AND SHIPPING REQUIREMENTS FOR SPECIFIC SAMPLE TYPES

5.1.1. HMW gDNA SAMPLES

HMW gDNA samples are best stored and shipped at 4°C. Please ship with ice packs or wet-ice (not frozen). Samples can also be shipped at room temperature as an alternative.

Freezing is not recommended because freeze–thaw cycles shorten the DNA.

However, if your HMW gDNA samples are already frozen, they should be shipped with dry ice or frozen ice packs.

5.1.2. PCR AMPLICONS AND SAMPLES IN 96-WELL PLATES

Short DNA fragments (PCR amplicons, cfDNA) should be shipped frozen on dry ice. See below for dry ice shipping requirements.

5.2. DRY ICE SHIPPING REQUIREMENTS

If shipping samples on dry ice, please adhere to the requirements listed below.

DO NOT FULLY SEAL	USE ENOUGH DRY ICE	AFFIX UN1845 LABEL
For safety, **dry ice must never be sealed** in a container with an airtight seal. A typical shipping container is an internal foam box with cardboard box exterior. To allow sublimating CO_2 to vent, **DO NOT completely tape the Styrofoam top or the box top**.	Samples must be shipped with a **minimum of 10 lbs (4.54 kg) of dry ice**. Dry ice sublimates at rate of 5–10 lbs per 24 hours. If you anticipate any delays during shipping (more than 24 hours), ensure that there is enough dry ice to sample integrity.	To comply with dangerous goods regulations, the outermost container must be labeled with a **hazard class 9 label** (**UN1845**), and a **net weight of dry ice** (in kilograms). 

5.3. SAMPLE SUBMISSION BY COURIER

5.3.1. BEFORE SHIPPING

Before shipping your sample, please make sure that your sample submission form has been reviewed and approved.

Please carefully follow shipping requirements for your sample type. These are listed on page 12.

5.3.2. WHERE TO SHIP

We recommend shipping Monday to Wednesday — we cannot accept packages on weekends.

Once your sample submission form is reviewed and approved, ship your samples to:

Dr. Andrew Mungall
Biospecimen Core, Room 508
Canada's Michael Smith Genome Sciences Centre
100 – 570 West 7th Avenue
Vancouver BC V5Z 1B3 Canada

email: amungall@bcgsc.ca
tel: 604-707-5900 ext. 675411

5.3.3. AFTER SHIPPING

Once samples have been shipped, please notify us of your shipment by email to GSC_Submissions@bcgsc.ca.

Make sure to include the shipment tracking number so we can monitor the progress during transit.

5.4. SAMPLE SUBMISSION BY DROP-OFF

5.4.1. BEFORE DROPPING OFF

Before dropping off your sample, please make sure that your sample submission form has been reviewed and approved.

When you are on your way to us with the sample, please email us at GSC_Submissions@bcgsc.ca so that we know when to expect you.

5.4.2. SAMPLE DROP-OFF AND PLATE PICKUP HOURS

9:30 – 11:30 am & 1:30 – 3:30 pm
Monday – Friday

5.4.3. ADDRESS

Canada's Michael Smith Genome Sciences Centre
100 – 570 West 7th Avenue
Vancouver BC V5Z 1B3 Canada

Once you arrive at our location, please call our office directly at 604-707-5900 ext. 675411.

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