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USER GUIDE

DNA SEQUENCING AT THE GSC

BC CANCER RESEARCH
Provincial Health Services Authority

CANADA'S MICHAEL SMITH GENOME SCIENCES CENTRE

Bringing Genomics to Life.

ABOUT US

The GSC's CAP, DAP 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 if you have any questions about the services we provide.

BC CANCER RESEARCH
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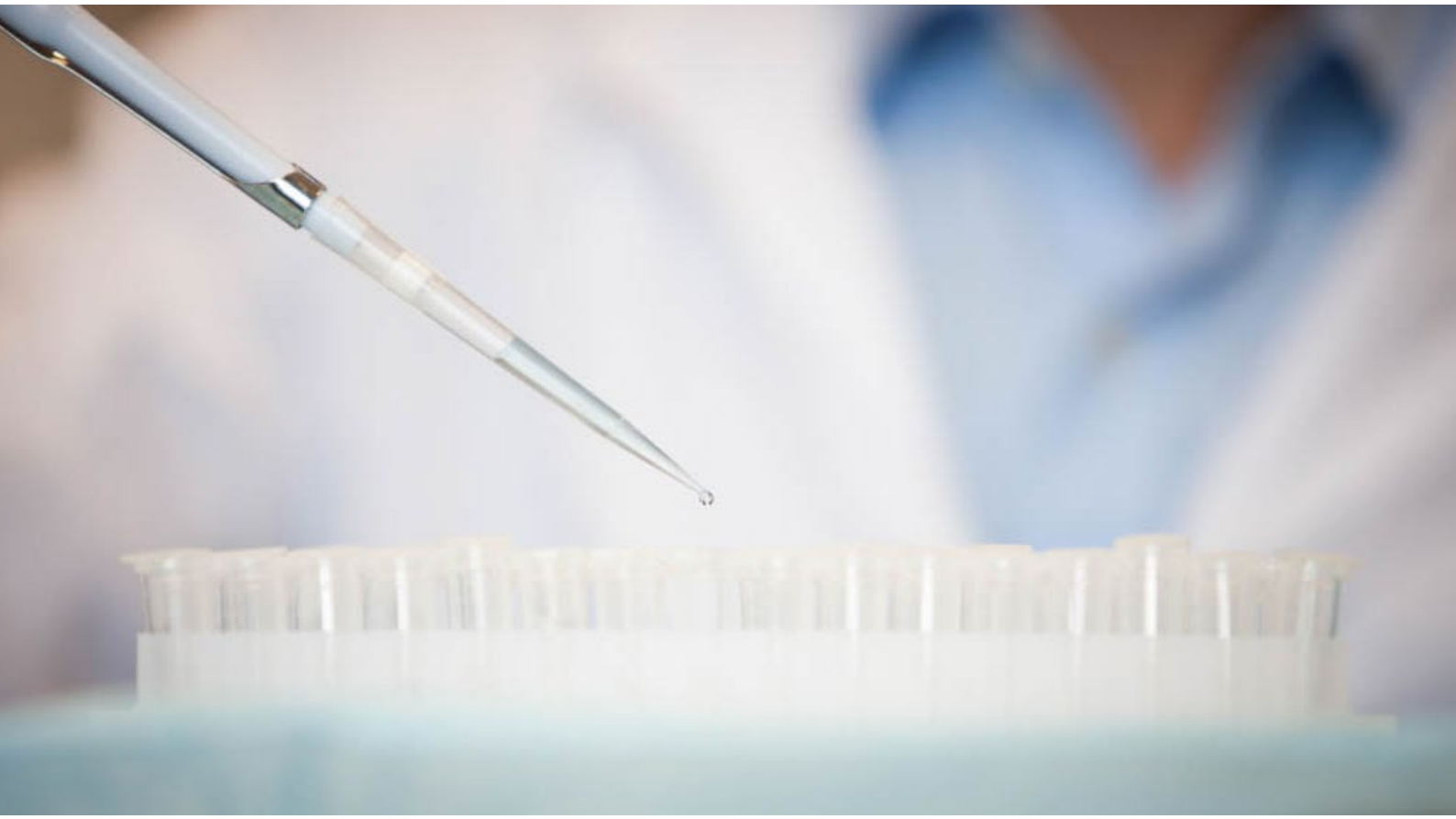
CANADA'S MICHAEL SMITH GENOME SCIENCES CENTRE

www.bcgsc.ca | info@bcgsc.ca | @BCCancer_GSC

CONTENT

THIS GUIDE

This user guide is for the preparation of samples for DNA extraction at the GSC or DNA extracted in your laboratory. If you are submitting constructed libraries, please refer to our Constructed Libraries User Guide.



1 SAMPLE PREPARATION

Submitting samples for DNA extraction at the GSC

We accept a variety of samples for DNA extraction and sequencing at the GSC including blood, fresh or frozen tissues, formalin-fixed paraffin-embedded (FFPE) tissues, and saliva and buccal (cheek) swabs. Tissue and cell samples are accepted in tube format All samples must be frozen at -80°C immediately after sectioning. The following starting material guidelines work well for human and mouse samples. If working with other organisms, please **contact us**.

SAMPLE TYPE	PIPELINE	SUBMISSION REQUIREMENTS
FFPE	Whole genome	Scrolls: min 120 mm^2 x 10 µm. For tissue surface area ≤ 120 mm^2 up to 3 scrolls per 1.0 mL matrix tube. Cores: 2.5 mm x 1-3 mm. Up to 2 cores per matrix tube.
OCT	Whole genome	Sections: 50 µm x 10 mm x 1 mm, minimum of 4 sections supplied in 2 mL tubes.
Fresh Frozen	Whole genome	Sections: 50 µm x 10 mm x 1 mm, minimum of 4 sections supplied in 2 mL tubes.
Cells	PCR-free whole genome	Minimum of 1 x 10^6 mammalian cells.

Samples for high molecular weight DNA extraction for Nanopore sequencing

SAMPLE TYPE	SUBMISSION REQUIREMENTS
Cell Culture	Submit 5-20 x 10^6 cells or more for HMW DNA extraction. Cells should be pelleted, washed with 1X PBS, supernatant removed, flash frozen in liquid nitrogen and stored at -80°C. Ship the cell pellets on dry ice.
Human/Animal Tissue	Fresh tissue must be snap frozen in liquid nitrogen right after harvesting and stored at -80°C. Frozen sample should be shipped on dry ice. Any freeze thaw cycles should be avoided. Please submit at least 100 mg of soft tissue that is minced by indicating the actual weight in mg. For potentially contaminated tissue such as intestine, please ensure guts are cleared out
Blood	Samples should be collected fresh with EDTA as anticoagulant. We prefer to receive fresh blood samples shipped over night on cold packs; these samples should be stored in a refrigerator temporarily. Blood samples can also be flash frozen and stored at -80°C. Frozen samples should be shipped on dry ice. 2 mL of mammalian blood sample will be required each for HWM DNA isolation. For sequencing over a larger number of flowcells additional material might be required.

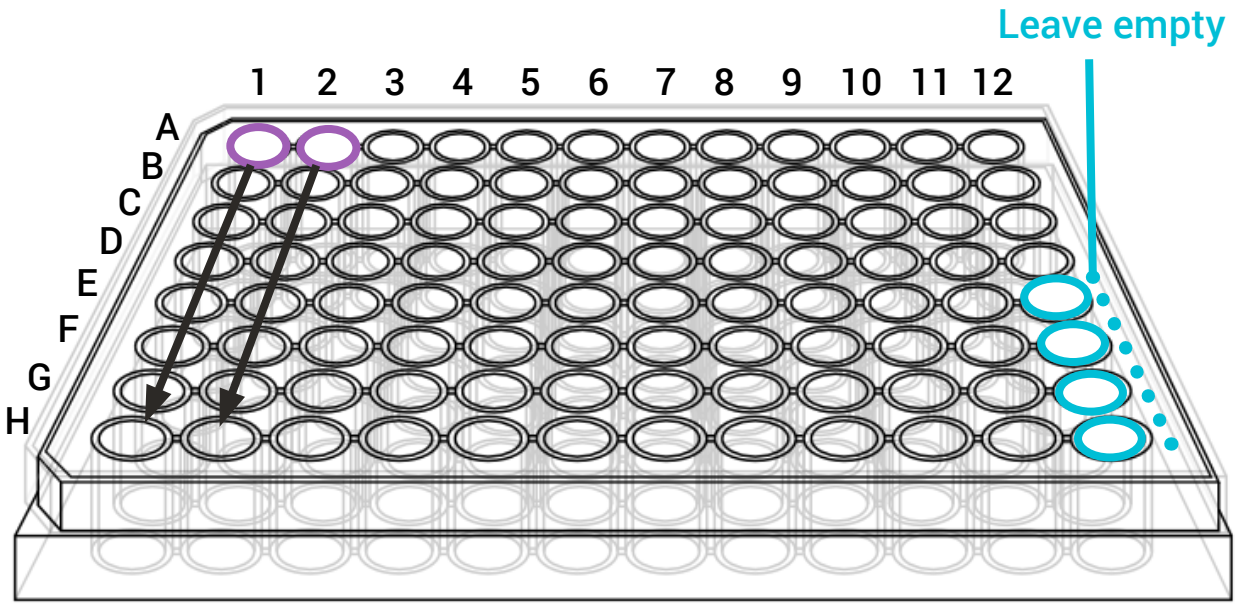
***Note** that we currently are not accepting tissue samples for nucleic acid extraction via online sample submission. Instructions for sample submission will be provided to you.

Submitting extracted DNA for library construction

Extracted DNA samples must be submitted frozen, and must be normalized by concentration. DNA samples are accepted in the following formats:

- Less than 24 samples: 1.5 mL snap-top Eppendorf tubes (or equivalent). Screw cap tubes are not accepted.
- 24 or more samples: Axygen 96 FS-C plates, or equivalent. If these are not available in your lab, contact us at GSC_submissions@bcgsc.ca and we will supply one.

If submitting in a 96-well plate format, arrange samples in columns (e.g., A1 to H1; A2 to H2). Wells E12, F12, G12 and H12 must be left empty for internal controls.



For best library construction results please submit the recommended amount of starting material or more. The recommended starting materials work well for human or mouse derived nucleic acids. If working with other organisms, please **contact us**.

LIBRARY TYPE	STARTING MATERIAL	SUBMISSION REQUIREMENTS
PCR-free genome	gDNA	**Recommended:** 1.0 µg **Minimum:** 700 ng **Concentration:** >17.5 ng/µL (in 25-40 µL) **Quantification method:** Quant-IT dsDNA HS assay **Quality assessment method:** Nanodrop **Quality value:** intact agarose gel **Additional assessment:** A260/280, A260/230
FFPE genomic	gDNA	**Recommended:** 1.0 µg **Minimum:** 500 ng **Concentration:** >12.5 ng/µL (in 25-40 µL) **Quantification method:** Quant-IT dsDNA HS assay **Quality assessment method:** Nanodrop **Additional assessment:** A260/280, A260/230
Genome shotgun low input (small gap)	gDNA	**Recommended:** 120 ng **Minimum:** 30 ng **Concentration:** >1.0 ng/µL (in 20-30 µL)
Circulating cell-free genome	gDNA	**Recommended:** 50 ng **Minimum:** 5 ng **Concentration:** in 10-30 µL
Bisulphite	gDNA	**Recommended:** 2.0 µg **Minimum:** 1.2 µg **Concentration:** >30 ng/µL (in 25-40 µL) **Quantification method:** Quant-IT dsDNA HS assay **Quality assessment method:** Nanodrop **Quality value:** intact agarose gel **Additional assessment:** A260/280, A260/230
Exome and special capture (small-gap genomic)	gDNA	**Recommended:** 1.0 µg **Minimum:** 500 ng **Concentration:** >12.5 ng/µL (in 25-40 µL) **Quantification method:** Quant-IT dsDNA HS assay **Quality assessment method:** Nanodrop **Quality value:** intact agarose gel **Additional assessment:** A260/280, A260/230
ChIP	gDNA	**Minimum:** 5-10 ng **Concentration:** >150 pg/µL (~5 ng in 35 µL) **Quantification method:** Quant-IT dsDNA HS assay **Quality assessment method:** PAGE if possible

Submitting extracted DNA for Nanopore sequencing

LIBRARY TYPE	STARTING MATERIAL	SUBMISSION REQUIREMENTS
PCR-free genome	DNA	**Recommended:** 6-50 µg **Minimum:** 5 µg **Concentration:** 100 ng/µL **Quantification method:** Qubit assay **Quality assessment method:** Nanodrop **Quality value:** A260/280 1.8-2.0; A260/230 2.0-2.2 **Additional assessment:** DNA must be dissolved in 10 mM TRIS (pH 8.0-8.4) (e.g. Qiagen EB Buffer)

2 SAMPLE SUBMISSION

Our Guide to Online Submissions will provide helpful information that will assist you in navigating our online submission website. Please contact us at GSC_Submissions@bcgsc.ca if you have any questions.

Online Submissions webpage: https://www.bcgsc.ca/samplesubmissions

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

This guide is for the submission of **extracted DNA**. If you are submitting samples for nucleic acid extraction at the GSC, submission instructions will be provided.

OVERVIEW

Please read the entire Overview section prior to starting your submission. This section will describe all information that is required to fill out your submission form. We recommend ensuring all required information is gathered prior to starting your submission.

To start a new submission, click on the **Dashboard** or **Submissions** tab on the left side panel, and then the "Start New Submission" button. Please note that an active Statement of Work (SOW) is required to start a new submission and that each individual SOW requires a new submission.

To continue working on an existing submission, click **Submissions** on the left side panel. This is the central location from which you can view all of your current and past submissions, as well as your submission status.

Questionnaire

There are three tabs to complete in this section, corresponding to three categories of information required. Please note that all the fields in the three tabs are mandatory. You need to complete each field in order to proceed to the next page.

1. SOW Selection
2. Sample Details
3. Work Request

Sample Information

There are ten mandatory tabs to complete by providing information about your samples. If submitting Xenograft, cDNA, Amplicons, ChIP or Cell Line samples there will be additional tab(s) to complete:

We recommend that you complete all the fields relevant to your samples. Some fields are mandatory (denoted by *) and other fields are optional (but recommended for tracking purposes).

1. Sample Information
2. Tissue Information
3. Pathology Information
4. Pathology Information Continued
5. Analyte Information
6. Analyte Information Continued
7. Work Request Assignment
8. Work Request Pooling
9. Comments and Extra Information
10. Submission Review

QUESTIONNAIRE

SOW section

- Submission Name: create a submission name that is complex enough to easily and uniquely identify each submission. For example, "Doe Lab ABC cell-line genomes."

- Statement of Work (SOW): select a SOW from the drop-down menu provided. If your SOW is not listed, please contact GSC Projects at **SOW@bcgsc.ca**. If a SOW has a revision associated, please select the parent SOW. Once selected, options for revisions will appear in a separate drop-down list. Please select the appropriate revision for the sample submission.

- Once the SOW is selected, PI and Dissemination Recipients(s) will be displayed on the right. To edit the dissemination recipients list, please return to the SOW homepage in the SOWs section. Only the PI or a designated Dissemination Editor can edit this list.

Sample details

- Sample Type: only one sample type is allowed per submission.

- Sample Number: a maximum of 92 samples, corresponding to one plate, are allowed per submission. Unless otherwise instructed, if submitting between 1-23 samples they need to be submitted in 1.5 mL Eppendorf tubes (or equivalent), and if submitting between 24-92 samples they need to be submitted in an Axygen 96 FS-C plate or equivalent. If this plate is not available in your lab, GSC will supply one. Please click the Request a Plate checkbox, and we will contact you to confirm whether you want to pick up the plate from the GSC or have it shipped. If the latter, you will need to provide your shipping address and FedEx account number.

- Taxonomy: you may enter multiple taxonomy IDs per submission. You may search by typing NCBI taxonomy ID (e.g. 9606) or name (e.g. human). Only selected taxonomy IDs will be available later in your submission.

- Additional Sample Information: select any that apply. There are additional fields you need to complete for the sample types listed.
- Unused Sample: indicate what you want us to do with any unused samples upon completion of your project. If you select “Destroy unused sample,” we will discard your samples six months following data dissemination.

Work request

- Please refer to your SOW to obtain the work request information that will be required in this section.
- If the samples you are submitting have different library protocol and/or sequencing goals, you will need to click “Add Additional Work Request” to be able to enter different goals for each sample.
- Once the sample information is completed and before the form is submitted, you will assign one of the work requests to each sample. At this point, you can also edit or add work request details.
- You will also be able to enter any additional comments regarding your work request such as specific pooling strategy, should extra or clarifying information be required.

SAMPLE INFORMATION

Overview

There are at least ten tabs to complete in this section. Note that mandatory fields are denoted by *. Certain field selections will grey out mandatory fields. In these situations field entry is not required.

For additional information on any given field, click the column heading (Figure 1, Arrow A).

Under each column heading are tools to help you complete each field more conveniently (Figure 1, Arrow B). Please refer to the tool tips on the top right corner to find out how each tool can help (Figure 2).

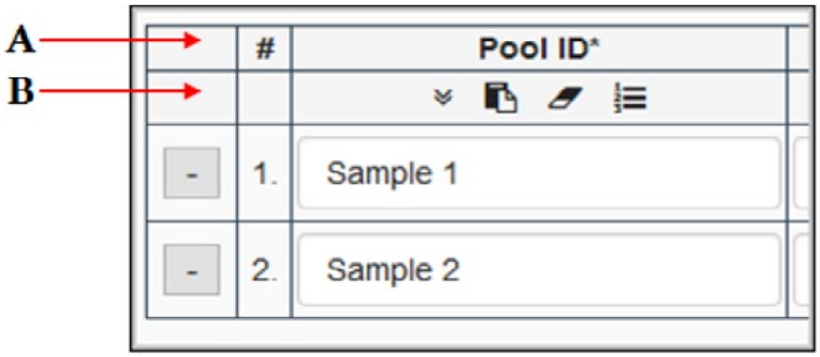


FIGURE 1

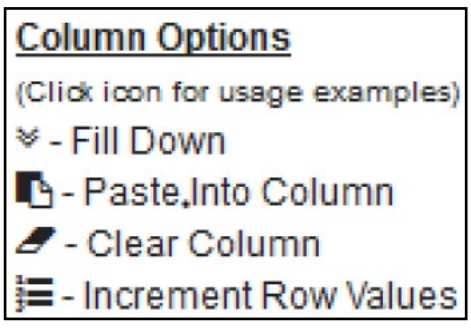


FIGURE 2

For each tab, you need to complete all the accessible mandatory fields before you can proceed to the next page. When you click the “Next” button, the webpage will validate each field to make sure all the cells meet our requirements. If there are any errors, cells will display red or orange error message(s). Please edit all the errors before proceeding to the next page. Please note that fields with warnings will be highlighted yellow for your review. It is not necessary to resolve warnings before proceeding.

Your draft submission will be saved when you click “Next”. Note that a submission can be saved at any step using the “Save as Draft” button at the bottom of the page.

You may add or remove a sample by clicking the  icon located on the top left corner, next to your submission name. Note that if you decrease the sample number by one, the last row will be deleted. If you decrease the sample number by two, the last two rows will be deleted.

Sample Information (fields vary slightly for non human samples)

- Sample ID*
- Alternate Sample ID
- Taxon*
- Tube Label* (when submitting 1-23 samples)
- Plate Location* (when submitting 24-92 samples): samples must be sorted by column (A1, B1, C1, etc. instead of A1, A2, A3, etc.).
- Anonymous Patient ID*: provide a minimum of five characters, recommended eight characters, with no symbols or spaces. Samples from the same patient must have the same Anonymous Patient ID. This is the only way to link samples collected from the same patient (e.g. normal/tumour samples).
- Participant Study ID

- Family Information
- Strain (for non-human samples)
- Developmental Stage
- Sex*
- Sample Collection Date

Tissue Information

- Anatomic Site*: this is a type-ahead search field. Please enter three or more characters to search and select the best option. If there are no appropriate matching selections, you may enter free text.
- Tissue Disease Status*: this field pertains to the tissue, not the patient. Please select from the drop-down menu provided. If “Normal” is selected, provide Disease Status of Patient (for Normal Samples) in the next column. If “Diseased” is selected, provide the pathology of the sample in the next page.
- Disease Status of Patient (for Normal Samples): this is a type-ahead search field. Please enter three or more characters to search and select the best option.
- Tissue Type*
- Cell Type
- Cell Line (for cell line samples)

Xenotransplant Recipient Information (For xenograft samples only)

- Xenograft*
- Recipient Taxonomy*
- Recipient Strain

Pathology Information

- Pathology*: this is a type-ahead search field. Please enter three or more characters to search and select the best option.
- Additional Pathology Information
- Pathology Occurrence*

Pathology Information - continued

- Grade
- Stage
- Tumour Content
- Treatment Status

Analyte Information

- Nucleic Acid Isolation Date
- Tissue Fixation Process*: this information is important for QC purposes. If FFPE is used, select “FFPE” under Tissue Fixation Process and “FFPE DNA” under Nature of Analyte.
- Nature of Analyte*
- Nucleic Acid Isolation Method

Analyte Information - continued

Prior to preparing your samples, review the table on page 7 to ensure your samples meet the requirements of amount, volume and concentration for submission.

- Volume (µL)*
- Concentration (ng/µL)*
- DNA Amount (ng)
- Storage Medium*
- Quantification Method*
- A260/280
- A260/230

cDNA or Amplicon Samples (For cDNA or Amplicon samples)

- RNA Source
- DNA/RNA Source
- Synthesis Method
- Amplicon Size
- Size Range

ChIP Samples (For ChIP samples)

- Crosslinking Method*
- Crosslinking Time (seconds)
- Sonication Time (seconds)*

- Antibody Used*

ChIP Samples - continued

- Antibody Catalogue Number
- Antibody Vendor
- Amount of Antibody Used (µg)
- Amount of Chromatin Used per IP (µg)

Work request assignment

Assign a work request to each sample. You may edit the Work Request by clicking  , located on the top right corner of the Work Request summary box. The required amount, volume and concentration are validated based on the library protocol selected. If you are submitting less than the minimum amounts, you will be asked to accept the risks of failure.

Work request pooling

Enter the number of samples per pool.

Comments and extra information (optional)

Use this field to provide any extra information or details regarding your submission or sample.

Submission review

AFTER SAMPLE SUBMISSION

The submission form will be reviewed for completeness and accuracy within two business days. You will then receive a notification of approval or a request for edits.

Once your submission has been approved, you will receive an email with detailed instructions for shipping your samples.

You can retrieve the submission info at any time from the submissions page under the Accepted tab.

If you have any questions during this process please contact the GSC submissions team at GSC_Submissions@bcgsc.ca.

3 SAMPLE SUBMISSION BY COURIER

Once the sample submission form is approved, samples must be shipped on dry ice and should be addressed to:

Dr. Andrew Mungall - Biospecimen Core, Room 508
Genome Sciences Centre BC Cancer
Suite 100 - 570 West 7th Avenue
Vancouver, BC Canada
V5Z 4S6

email: amungall@bcgsc.ca

Tel: 604-707-5900 ext 3251

When samples have been shipped, we ask that you please email **sampleshipments@bcgsc.ca** to notify us of your shipment and the associated tracking number so we can monitor the progress during transit.

Please ensure that there is sufficient dry ice for a couple of days. We recommend shipping Monday to Wednesday as we cannot accept packages on weekends.

4 SAMPLE DROP-OFF

The sample submission form must be reviewed and approved by GSC personnel prior to submitting samples to the GSC.

Regular hours for sample drop-off and plate pick-up:

Monday – Friday: 9:30-11:30 am and 1:30-3:30 pm

Location:

Suite 100-570 West 7th Avenue, Vancouver, BC V5Z 4S6

To enter the building, dial #100 on the intercom and the receptionist will let you in. The reception is on the ground floor (past the elevators and on the left). Go through to reception and ask the receptionist to call or page anyone from the Biospecimen Core group. We'll come down to reception to meet you.

Technology platform

Echelon Innovation Centre
570 West 7th Avenue
Vancouver, B.C. V5Z 4S6
Phone: 604-707-5900

Research department

BC Cancer Research Centre
675 West 10th Avenue
Vancouver, B.C. V5Z 1L3
Phone: 604-675-8000

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