

# USER GUIDE



## RNA SAMPLES



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.



[www.bcgsc.ca](http://www.bcgsc.ca) | [info@bcgsc.ca](mailto:info@bcgsc.ca) | [@BCCancer\\_GSC](https://twitter.com/BCCancer_GSC)

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# | THIS GUIDE

This user guide is for the preparation and submission of extracted RNA. If you are submitting DNA, constructed libraries, cells or bodily fluids, please refer to the user guides on [our website](#).

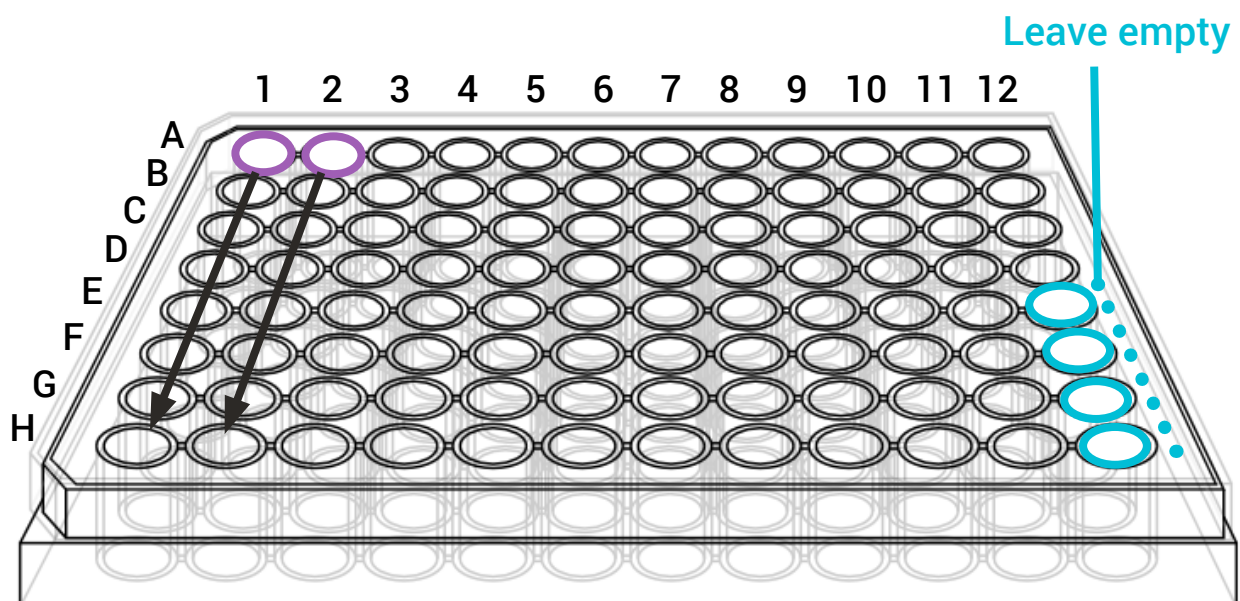
**\*Note:** tissue samples are currently not supported by our online submission portal. Submission instructions will be provided to you.

# 1 SAMPLE PREPARATION

## Submitting extracted RNA for library construction

Extracted RNA samples should be submitted frozen, and samples must be normalized by concentration. RNA 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, please see instructions on [page 9](#) for requesting a plate from us.
- If submitting in a 96-well plate format, arrange samples in columns (eg. 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
mRNA using strand specific protocols (ssRNA-Seq)	Total RNA	<b>Recommended:</b> 750 ng <b>Minimum:</b> 250 ng (enquire for non-mammalian) <b>Concentration:</b> >12.5 ng/μL (in 20-35 μL) <b>Quantification:</b> Agilent Bioanalyzer RNA nano chip <b>Quality assessment:</b> Agilent Bioanalyzer RNA nano chip <b>Quality value:</b> RIN > 7 (not applicable for FFPE) <b>Additional assessment:</b> A260/280, A260/230
Low input RNA (ribodepletion)	Total RNA	<b>Recommended:</b> 50 ng <b>Minimum:</b> 40 ng <b>Concentration:</b> >4 ng/μL (in 10 μL) <b>Quantification:</b> Agilent Bioanalyzer RNA pico chip <b>Quality assessment method:</b> Agilent Bioanalyzer RNA pico chip <b>Quality value:</b> RIN > 7 (not applicable for FFPE) <b>Additional assessment:</b> A260/280, A260/230
Ribodepleted strand specific RNA-Seq	Total RNA	<b>Recommended:</b> 200 ng (1 μg for FFPE RNA) <b>Minimum:</b> 125 ng (800 ng for FFPE RNA) <b>Concentration:</b> >12.5 ng/μL (>80 ng/μL for FFPE) (in 10 μL) <b>Quantification:</b> Agilent Bioanalyzer RNA nano chip <b>Quality assessment method:</b> Agilent Bioanalyzer RNA nano chip <b>Quality value:</b> RIN > 7 (not applicable for FFPE) <b>Additional assessment:</b> A260/280, A260/230
miRNA	Total RNA	<b>Recommended:</b> 1 μg <b>Minimum:</b> 700 ng <b>Concentration:</b> >70 ng/μL (in >4 μL) <b>Quantification:</b> Agilent Bioanalyzer RNA nano chip <b>Quality assessment method:</b> Agilent Bioanalyzer RNA nano chip <b>Quality value:</b> RIN > 7 (not applicable for FFPE) <b>Additional assessment:</b> A260/280, A260/230
Full length mRNA direct sequencing (nanopore)	Total RNA	<b>Recommended:</b> 50-100 μg <b>Minimum:</b> 20 μg <b>Concentration:</b> >200 ng/μL <b>Quantification:</b> Agilent Bioanalyzer RNA nano chip <b>Quality assessment method:</b> Agilent Bioanalyzer RNA nano chip <b>Quality value:</b> RIN > 7

## 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](mailto:GSC_Submissions@bcgsc.ca) if you have any questions.

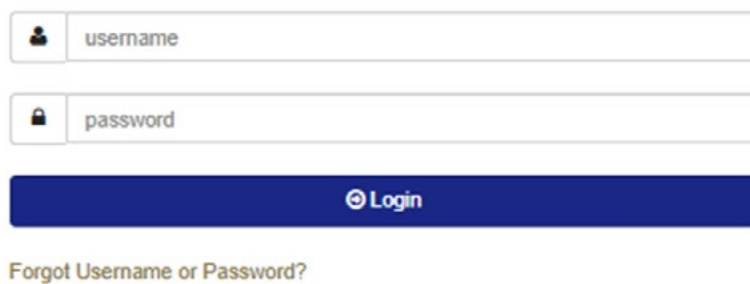
Online Submissions webpage: <https://www.bcgsc.ca/samplesubmissions>

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

## GETTING STARTED

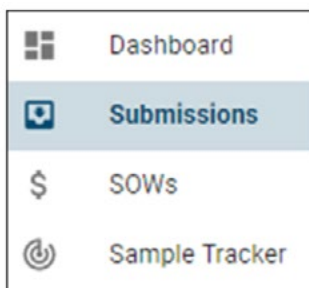
1. You can access the GSC Sample Submission Interface by entering the link in Firefox or Google Chrome: <https://www.bcgsc.ca/samplesubmissions>
2. Enter your username and password, and then click Login:



### GSC Sample Submission Interface



The screenshot shows the login interface for the GSC Sample Submission Interface. It features two input fields: the first is labeled 'username' and contains a person icon; the second is labeled 'password' and contains a lock icon. Below these fields is a dark blue button with a white 'Login' label and a circular arrow icon. Underneath the button is a link that reads 'Forgot Username or Password?'.

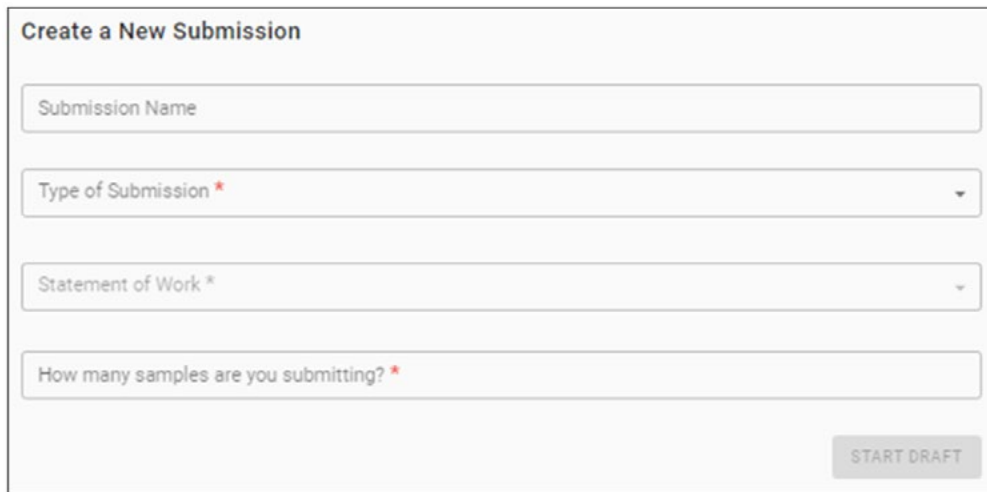
3. To start a new submission, click Dashboard or Submissions on the left side panel:



- Click the  (Dashboard) icon on the left side panel, and then click Start New Submission under Quick Links, or
- Click the  (Submissions) icon on the left side panel, and then click CREATE NEW SUBMISSION located on the top right corner.



## Create New Submission / SET UP



1. Submission Name: This is an optional field to help you identify your submission. Create a submission name that is complex enough to easily and uniquely identify each submission.
2. Type of Submission: Select from the drop-down menu. Only one sample type is allowed per submission.
3. Statement of Work: Select from the drop-down menu. Once selected, PI, Dissemination Recipient(s), and Scope of Work will be displayed. To make any changes to this information, please contact [SOW@bcgsc.ca](mailto:SOW@bcgsc.ca).
4. Sample Number: A maximum of 92 samples, corresponding to one 96-well plate (four wells are reserved for GSC controls), is allowed per submission. Unless otherwise instructed, 1-23 samples must be submitted in 1.5 mL Eppendorf tubes and 24-92 samples must be submitted in an Axygen 96 FS-C plate.

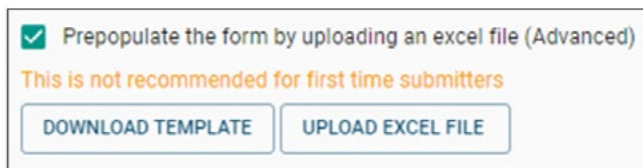
If an Axygen 96 FS-C plate or equivalent is not available in your lab, a pre-barcoded plate can be supplied by the GSC. For this request, click "Yes" under Request a plate:



We will contact you when the plate is ready to be picked up from the GSC or shipped to your lab. If the latter, we will ask you to provide your shipping address and FedEx account number.

5. Taxonomy: You may enter multiple Taxonomy IDs per submission. Search for the taxa of your samples by typing NCBI Taxonomy ID (e.g., 9606) or name (e.g., human). Only selected Taxonomy IDs will be available later in the SAMPLE INFORMATION section under the Taxonomy field.
6. Sample Options: Select all that apply to your samples (Cell Lines, and/or Xenograft). For these sample types, there will be additional fields to complete.
7. Prepopulate the form by uploading an Excel file: Advanced users (i.e., those who often use the GSC Sample Submission Interface) have an option to prepopulate the sample information form by uploading an Excel file. This is not recommended for first time submitters.

- Click the checkbox next to Prepopulate the form by uploading an excel file:



Prepopulate the form by uploading an excel file (Advanced)  
This is not recommended for first time submitters  
DOWNLOAD TEMPLATE    UPLOAD EXCEL FILE


- Click DOWNLOAD TEMPLATE.
- Enter your sample information directly into the Excel file that was generated:
  - » You can copy and paste entries from a pre-existing Excel file that contains your sample information.
  - » If a field has a drop-down menu, you must select from the available options.
  - » Some fields may require validation after the sample information form has been populated.
  - » All fields do not need to be completed in order to use this feature to prepopulate the sample information form.

- » Any missing or invalid fields can be corrected within the actual sample information form.
  - Once completed, save the Excel file on your computer.
  - Go back to the Create a New Submission section and click UPLOAD EXCEL FILE.
  - A dialogue window will open that allows you to navigate to the location on your computer where you saved the Excel file.
  - The SAMPLE INFORMATION section will be auto-populated based on the entries in the Excel file.
8. 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.
9. Click START DRAFT on the bottom right corner.
10. You can always go back to the SET UP section to edit the above fields:



## SAMPLE INFORMATION FORM

### Overview

1. Mandatory fields are denoted by \*. Certain field selections will grey out mandatory fields. In these situations, field entry is not required. For example, if Tissue Disease Status is "Normal," Pathology will be greyed out, so you do not need to provide this information.
2. For additional information on any given field, click the  icon next to the column heading:




3. There are tools on the top right corner to help you complete each field more conveniently:



- Paste Range: Paste Range can be helpful when you have a pre-existing Excel file that you would like to copy and paste line items from. For the fields without drop-down menu, you can copy and paste directly into the form from your Excel file without using this tool.
- Fill Down: Fill Down can be helpful when you have several consecutive rows that will contain the same value.
- Clear Column: Clear Column can be helpful when you would like to reset values in the entire column.

- Validator will give an orange warning message for any entries that we want you to double-check. If the information looks correct, you can proceed as is. You will receive a red error message when the entry is missing or incorrect. These entries must be corrected before you can submit your sample information form:



- You can download the Excel file of your submission by clicking the  (Download) icon next to Submission ID:

Submission	Name
 847	Download Excel

## Sample Information

- Sample ID: Sample ID is a unique identifier referring to the actual sample being submitted. You will receive your data back linked to Sample ID. Sample ID needs to be:
  - Between five and 80 characters with no symbols and no spaces (hyphen and underscore are allowed).
  - Sufficiently complex to help distinguish your samples from other samples.
  - If submitting two or more identical samples, indicate that they are identical by using the same Sample ID.

2. Tube Label for 1-23 samples: Unless otherwise instructed, ensure that your samples are submitted in 1.5 mL Eppendorf tubes. Enter Tube Label as it is written on the tube. This can be the same as the Sample ID. Tube Label needs to be:
  - Less than 13 characters with no symbols (hyphen and underscore are allowed).
  - All labels must be unique to differentiate between tubes.
3. Plate Location for 24-92 samples: Unless otherwise instructed, ensure that your samples are submitted in an Axygen 96 FS-C plate or equivalent. Samples must be sorted by columns, so they are A1, B1, C1, etc. instead of A1, A2, A3, etc. If samples are not sorted by columns, additional costs may apply.

You can click ASSIGN PLATE LOCATION on the top right corner to automatically populate the Plate Location field. Please note that wells E12, F12, G12, H12 are reserved for the GSC internal control samples and cannot be assigned.

4. Taxonomy ID: Auto-filled from the SET UP section.
5. Patient ID: This is used as a common identifier in our database to link all samples received from the same patient; this assists with our QC processes as well as downstream analysis. Patient ID needs to be:
  - A minimum of five characters with no symbols and no spaces (hyphen and underscore are allowed).
  - Sufficiently complex to avoid unrelated individuals from having the same ID.
  - De-identified; do not use hospital ID or PHN which may link back to the patient or patient records.
  - If two or more samples are from the same patient, they must have the same Anonymous Patient ID; do not add a prefix or suffix to differentiate normal/tumor pairs or different aliquots of the same sample.

6. Family Information: If applicable, enter any relevant family information such as a family history of disease or specific family relationship to other samples being submitted (e.g., proband, father, mother, sibling).
7. Developmental Stage: Enter the developmental stage of the sample being submitted (e.g., larvae, embryo, adult, pediatric).
8. Strain: If applicable, enter the strain (e.g., mouse strain).
9. Sex: Select from the drop-down menu. This information is helpful for QC processes and downstream analysis.
10. Collection Date: Enter the date that your sample was collected using the calendar (e.g., date of patient blood draw or date that a sample was collected in the field). The date must be in the YYYY-MM-DD format (e.g., 2020-01-24).
11. Anatomic Site: Enter the site from which the sample you are submitting was derived (e.g., peripheral blood, liver). This is a type ahead input field. Begin typing the anatomic site and a list of suggestions will appear for selection. If necessary, you may enter free text. An orange warning message will display, but you can still proceed.
12. Tissue Disease Status: Select from the drop-down menu.
13. Disease Status of Patient (for Normal Samples): Enter the disease/health status of the patient. This information can help us track the disease status of the patient separately from the sample. For example, if you are submitting a normal blood sample from a patient with breast cancer, the sample is normal but the disease/health status of the patient is "Breast Cancer".
14. Tissue Type: For diseased samples, select from the drop-down menu. Select "Benign" for benign tumour and "Non-neoplastic disease" if not cancer.
15. Cell Type: If a specific cell type has been used, specify the cell type. For example, if

B-cells were sorted from lymph node, Anatomic Site would be “Lymph node” and Cell Type would be “B-cells.” This is a type ahead input field. Begin typing the cell type and a list of suggestions will appear for selection. If necessary, you may enter free text. An orange warning message will display, but you can still proceed.

16. Pathology: This is a type ahead input field. Begin typing the disease name. A list of suggestions will appear for selection. The list is generated from <http://disease-ontology.org>. If there are no appropriate matching selections, you may enter free text. Do not use abbreviations. An orange warning message will display, but you can still proceed.

17. Additional Pathology Info: For diseased samples, if applicable, enter pathology subtype/subgroup information such as molecular subgroup.

18. Pathology Occurrence: For tumour samples, select from the drop-down menu.

19. Pathology Grade: For tumour samples, if known, enter grade (e.g., G1, G2, G3, G4, Low, High).

20. Pathology Stage: For tumour samples, if known, enter stage by selecting from the drop-down menu.

21. Treatment Status: Select treatment status at the time your sample was collected from the drop-down menu.

22. Nucleic Acid Isolation Date: Enter the date your RNA was isolated in the YYYY-MM-DD format.

23. Tissue Fixation Process: Provide information if any fixative was used from the drop-down menu. Select “None” if not applicable.

24. Nature of Analyte: Select from the drop-down menu.

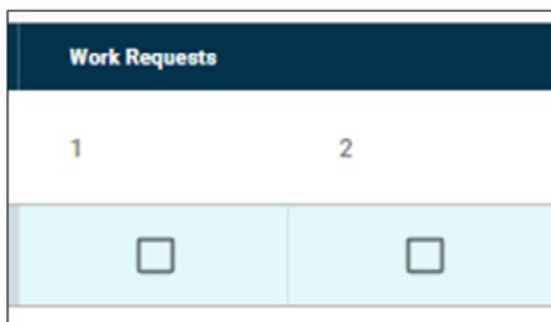
25. Isolation Method: Enter the method used to isolate your nucleic acid. If a kit was



- used, provide the name and the manufacturer of the kit (e.g., Qiagen AllPrep).
26. DNase Treatment Method Applied: Select from the drop-down menu.
  27. Volume (uL): Volume of nucleic acid that you are submitting in uL – Do not enter the unit. Prior to preparing your samples, review the table on [page 6](#) to ensure that your samples meet the requirements of volume, concentration, and amount for submission.
  28. Concentration (ng/uL): Concentration of nucleic acid that you are submitting in ng/uL – Do not enter the unit. Prior to preparing your samples, review the table on [page 6](#) to ensure that your samples meet the requirements of volume, concentration, and amount for submission.
  29. Amount (ng): Auto-calculated from Volume and Concentration.
  30. Storage Medium: This is a type ahead input field. Begin typing the storage medium and a list of suggestions will appear for selection. If necessary, you may enter free text. An orange warning message will display, but you can still proceed. If the storage medium is TE, specify the molarity (e.g., 10:1 or 10:0.1).
  31. Quantification Method: Select the method used to quantify your samples from the drop-down menu.
  32. RNA Quality Test: Select the method used to assess the quality of your samples from the drop-down menu. Select “N/A” if quality was not tested.
  33. RNA Quality Test Value: Enter quality score of RNA you are submitting.

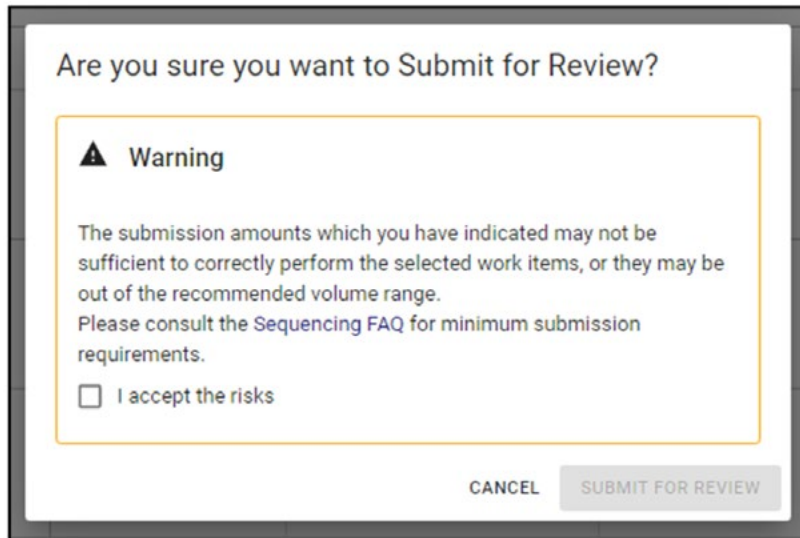
## WORK REQUESTS & WORK REQUEST ASSIGNMENT

1. Please refer to your SOW to obtain the work request information that will be required in this section.
2. If the samples you are submitting have different library protocols and/or sequencing goals, you will need to click ADD WORK REQUEST on the top left corner to be able to enter different work requests for each sample.
3. Once the WORK REQUESTS section has been completed, you will need to assign one of the work requests to each sample in the WORK REQUEST ASSIGNMENT section.
4. Assign a work request to each sample by clicking the checkbox under the appropriate Work Requests column:



Work Requests	
1	2
<input type="checkbox"/>	<input type="checkbox"/>

- You can edit work request details by going back to the WORK REQUESTS section.
  - If the same work request should be applied to all the samples, simply click the first checkbox, then click FILL DOWN on the top right corner.
5. The required volume, concentration, and amount 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 before you can submit your sample information form for review:

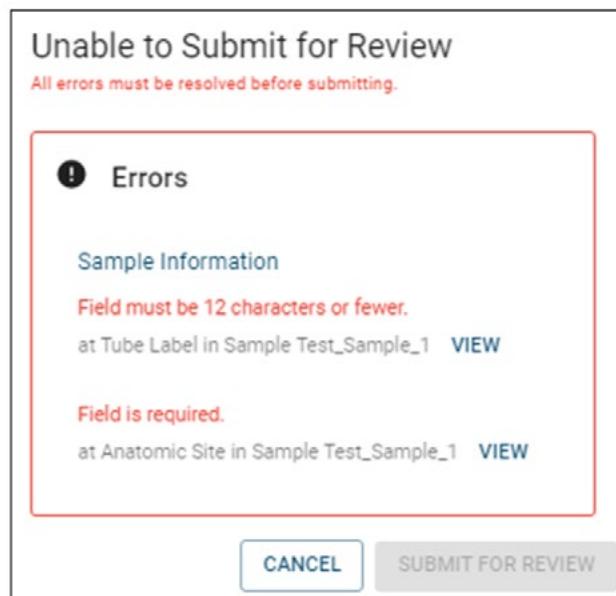


## COMMENTS & PLATE INFORMATION

1. Use the COMMENTS section to:
  - Enter any additional comments regarding your work request, such as specific pooling strategy, should any clarifying information be required.
  - Provide any extra information or details regarding your submission or samples.
2. If you are submitting 24-92 samples, you will also need to provide Plate ID and Rac ID (if applicable) in the PLATE INFORMATION section:
  - Plate ID is the name your lab assigns to the plate. You may even handwrite your SOW number, project name, etc. on the plate and use that as Plate ID. If your samples are being submitted on a pre-barcoded plate provided by the GSC, Plate ID may not be identical to Rac ID, as Rac ID is the internal name used by the GSC.
  - If you have received a pre-barcoded plate from the GSC, please enter the Rac ID affixed to the plate. If you have not received the plate yet, you may complete the submission without this information. You will be prompted to enter the information later.

## Submitting the Sample Information Form

1. Once you have reviewed all the fields and are satisfied with the sample information form, click SUBMIT FOR REVIEW on the top right corner.
2. If any of the samples do not meet the minimum recommended amount, click on the “I accept the risks” checkbox in order to proceed.
3. If there are any errors that need to be addressed, you will see the following message. Click VIEW and you will be taken directly to the cell whose error must be fixed before submitting.






4. Once your sample information form has been successfully submitted, you will receive “Sample Information Form Received for Review” email notification.

## After Submitting Sample Information Form for Review

1. Your sample information form will be reviewed for completeness and accuracy. You will then receive “Edits Requested” or “Submission Approved” email notification.
2. Edits Requested:
  - In order to resubmit the form, you **must confirm or amend** all of the fields which have been highlighted yellow or orange by the reviewer. As you address each field in the form, its colour will change to green or blue. **When there are no more yellow or orange fields left, you can resubmit.**
  - Some fields may also contain a comment made by the reviewer, in which case those fields will be marked with a small red triangle. You can hover your mouse over those fields to see the reviewer comments.
  - You also have the option of making edits to any field, including those which have not been highlighted by the reviewer. Any such fields will become highlighted purple, and are subject to final review by the GSC Submission team.
  - Here is a legend of the highlighting colours that you may encounter while making edits:
    - » Yellow: **Needs Attention**; the reviewer believes an edit may be required.
    - » Orange: **Changed by Reviewer**; the reviewer has already changed the value of this field, and would like you to confirm (or re-edit). Hover over the field with your mouse to see the change that has been made.
    - » Blue: **Amended by Submitter**; to be reviewed by the GSC Submission team.
    - » Green: **Confirmed by Submitter**; subject to final review by the GSC Submission team.
    - » Purple: **Additional Edit by Submitter**; subject to final review by the GSC Submission team.

- There are three modes that you should use while addressing reviewer comments and changes:



- »  **Edit:** This is the default mode. When in 'Edit' mode, you are able to alter any field to change its value. Whenever you have made an edit to a field, it will turn blue or purple.
- »  **Highlight:** When you switch to 'Highlight' mode, clicking on a field should turn it green. You may highlight a field green to indicate that you agree with an edit made by the reviewer, or that you believe the highlighted field already has the correct value. (Clicking on a field multiple times while in highlight mode will toggle between 'unconfirmed' and 'confirmed' state).
- »  **Read-only:** All error and warning messages will be hidden.

3. Once your submission has been approved, you will receive a "Submission Approved" email notification with detailed instructions for dropping off or shipping your samples.

3

## SAMPLE SUBMISSION BY COURIER

Once the sample submission form is approved, samples should be shipped on dry ice and 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 (\*If shipping via Purolator, use V5Z 1B3)

email: [amungall@bcgsc.ca](mailto:amungall@bcgsc.ca)

Tel: 604-707-5900 ext 673251

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

If shipping on dry ice, 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