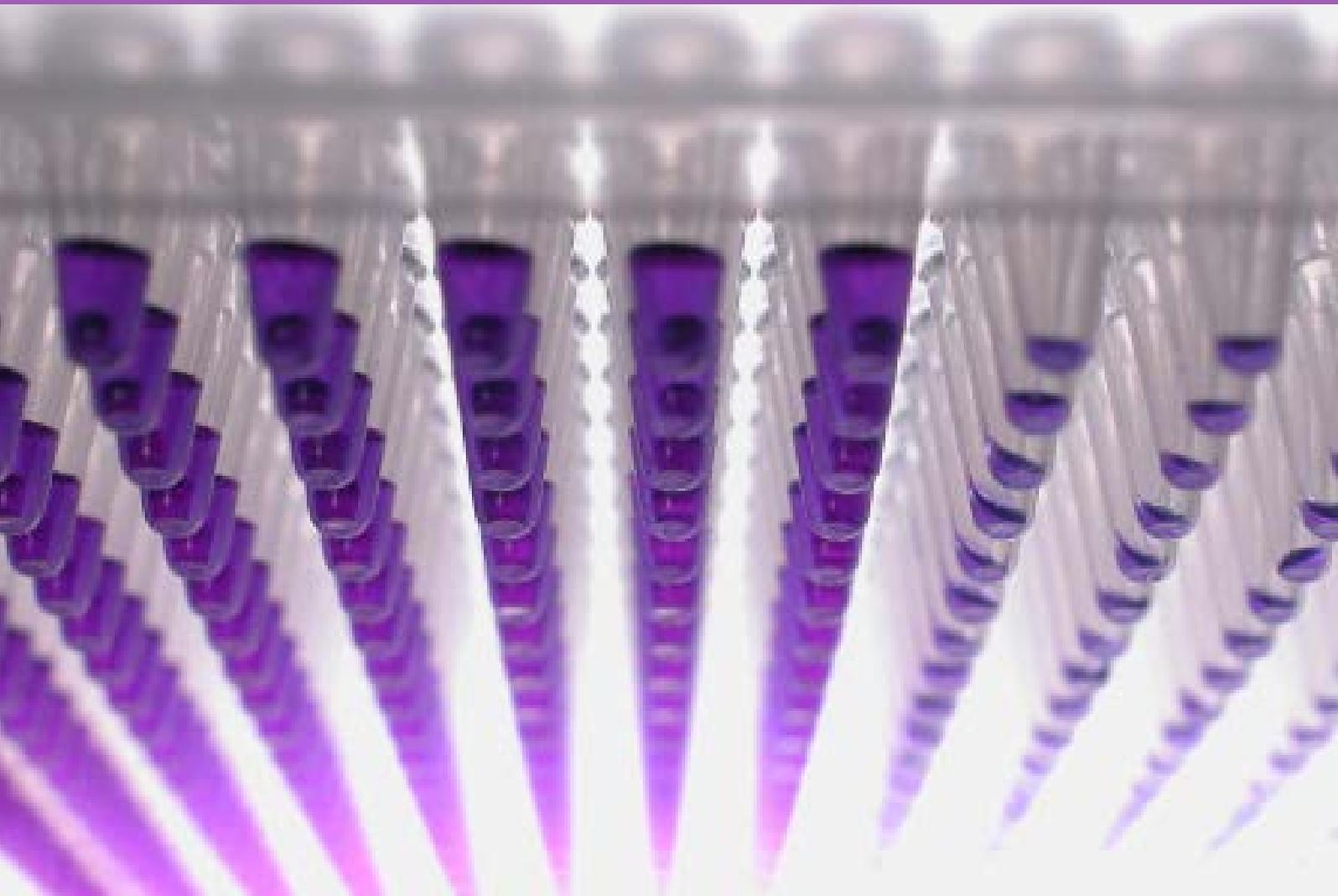


USER GUIDE



METAGENOMES



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 | 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 **metagenome** samples. If you are submitting a different sample type, please refer to the other guides on [our website](#).

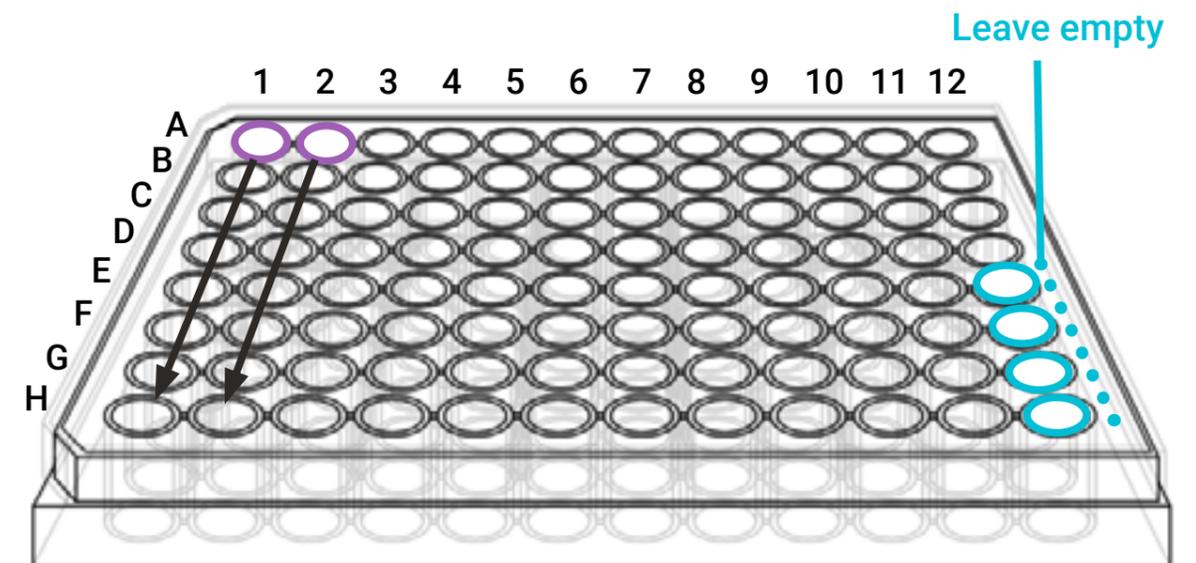
1 SAMPLE PREPARATION

SUBMITTING METAGENOMES FOR LIBRARY CONSTRUCTION

Extracted nucleic acids must be submitted frozen and must be normalized by concentration. 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 10](#) for requesting a plate from us.

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 PROTOCOL	STARTING MATERIAL	SUBMISSION REQUIREMENTS
PCR-Free Genome	Genomic DNA (gDNA) or Total Nucleic Acid (TNA)	Recommended: 1.0 µg Minimum: 700 ng Concentration: >17.5 ng/µL (in 25-40 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2
FFPE Genome	gDNA or TNA	Recommended: 1.0 µg Minimum: 500 ng Concentration: >12.5 ng/µL (in 25-40 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2
Exome and Specific Capture (FFPE)	gDNA or TNA	Recommended: 1.0 µg Minimum: 500 ng Concentration: >12.5 ng/µL (in 25-40 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230)
Genome Low Input	gDNA or TNA	Recommended: 120 ng Minimum: 25 ng Concentration: >1.0 ng/µL (in 10-25 µL)
Exome and Specific Capture (Genome Low Input)	gDNA or TNA	Recommended: 120 ng Minimum: 25 ng Concentration: >1.0 ng/µL (in 10-25 µL)
Circulating Cell-Free Genome	Cell-free DNA (cfDNA)	Recommended: 50 ng Minimum: 5 ng Concentration: >0.16 ng/µL (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 or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2

LIBRARY PROTOCOL	STARTING MATERIAL	SUBMISSION REQUIREMENTS
Exome and Specific Capture (Small-Gap)	gDNA or TNA	Recommended: 1.0 µg Minimum: 500 ng Concentration: >12.5 ng/µL (in 25-40 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2
Genome Tagmentation	gDNA or TNA	Recommended: 120 ng Minimum: 30 ng Concentration: >1.0 ng/µL (in 20-30 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2
Exome and Specific Capture (Tagmentation)	gDNA or TNA	Recommended: 120 ng Minimum: 30 ng Concentration: >1.0 ng/µL (in 20-30 µL) Quantification method: Quant-IT or Qubit dsDNA HS assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2

SUBMITTING EXTRACTED DNA FOR NANOPORE SEQUENCING

For long-read nanopore sequencing of genomic high molecular weight DNA extracted from frozen tissues, cells or bodily fluids, we strongly recommend size-selection to deplete DNA molecules <15 kb in length. We use a Blue Pippin instrument (Sage Bioscience) for this size-selection with 5 µg DNA input in 30 µL volume. For samples in which 5 µg is not available, size-selection can be bypassed if the submitter accepts the risk of shorter N50 read lengths and sequencing yields from the PromethION. In such cases, 2 µg of high molecular weight DNA should be provided with a minimum concentration of 50 ng/µL.

LIBRARY PROTOCOL	STARTING MATERIAL	SUBMISSION REQUIREMENTS
PCR-Free Genome	High Molecular Weight (HMW) DNA or gDNA	Recommended: 10 µg Minimum: 5 µg Maximum: 50 µg Concentration: >167 ng/µL (in 30 µL) Quantification method: Qubit assay Quality assessment method: Spectrophotometry (A260/280, A260/230) Quality value: A260/280 ratio 1.8-2.0; A260/230 ratio 2.0-2.2

2 ONLINE SAMPLE SUBMISSION

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

Our “Online Submissions Webpage” can be found [here](#). Please use Firefox or Google Chrome browsers.

This guide to online submissions will provide helpful information that will assist you in navigating our online submission website.

Please write to GSC_Submissions@bcgsc.ca if you have any questions.

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:

The screenshot shows a login interface with the following elements:

- A label "Username" above a text input field.
- A label "Password" above a text input field.
- A link "Forgot Password?" located to the right of the password field.
- A blue button labeled "Log In" at the bottom of the form.

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.

HOW TO CREATE A NEW SAMPLE SUBMISSION

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.
4. Sample Number: A maximum of 92 samples, corresponding to one 96-well plate, is allowed per submission (4 wells are reserved for GSC controls). 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. 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:

- 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.
6. 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.
 7. Click "START DRAFT" on the bottom right corner.
 8. 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:

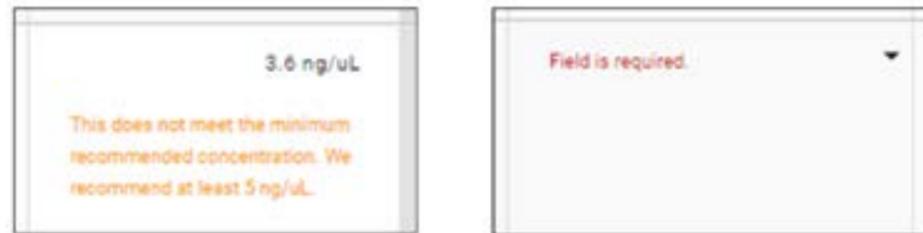


3. There are tools on the top right corner to help you complete each field more conveniently:



- **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:



Sample Information

- Sample ID: This field is a unique identifier referring to the actual sample being submitted; you will receive your data back linked to Sample ID.

Sample ID Requirements:

- 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; and
- If submitting two or more identical samples, indicate that they are identical by using the same Sample ID.

- 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 Requirements

- Less than 13 characters with no symbols (hyphen and underscore are allowed); and
- All labels must be unique to differentiate between tubes.

- 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.

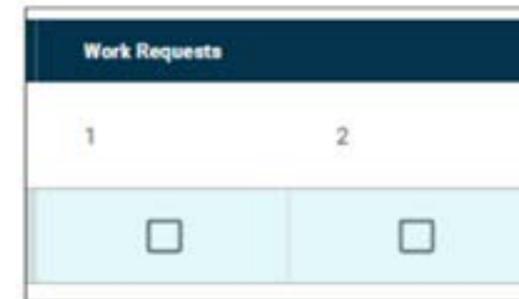
- Taxonomy For Alignment: This is an optional field. Begin typing the taxonomy for alignment and a list of suggestions will appear for selection.
- Alternative Sample ID: Alternative Sample ID is an optional identifier. It needs to contain no symbols or spaces (hyphen and underscore are allowed) and be sufficiently complex to help distinguish your samples from other samples.
- Nature of Analyte: Select from the drop-down menu.
- 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.

8. Concentration (ng/ μ L): Concentration of nucleic acid that you are submitting in ng/ μ L – 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.
9. Amount (ng): Auto-calculated from Volume and Concentration.
10. 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 molar ratio (e.g., 10:1 or 10:0.1).
11. Quantification Method: Select the method used to quantify your samples from the drop-down menu. Note that Qubit is the preferred quantification method.

WORK REQUESTS & WORK REQUEST ASSIGNMENT

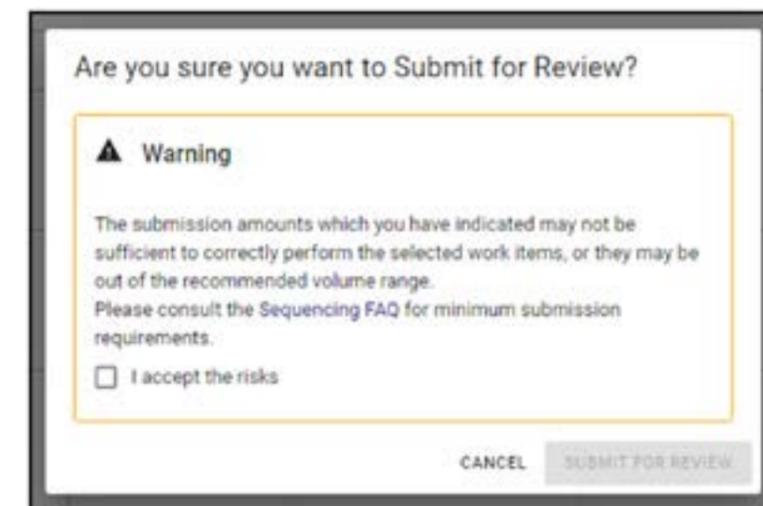
1. Please refer to your SOW to obtain the work request information that will be required in this section.
2. If the samples you are submitting have different library protocols and/or sequencing goals, you will need to click “ADD WORK REQUEST” on the top left corner to be able to enter different work requests for each sample.
3. Enter any additional comments regarding your work request in the “Comments” field such as pooling strategy.
4. 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.

5. Assign a work request to each sample by clicking the checkbox under the appropriate Work Requests column:



- 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.

6. 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:

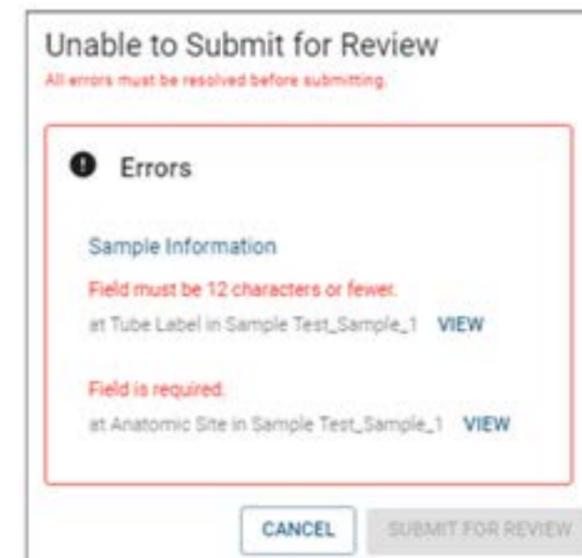


COMMENTS & PLATE INFORMATION

1. Use the “COMMENTS” section to provide any extra information or details regarding your submission of 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.

For "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; and
- 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.

Below 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.

You should use the following three modes ("Edit", "Highlight" and "Read-only") when 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.

2. 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

Tel: 604-707-5900 ext 675411

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.

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

Please email us at GSC_Submissions@bcgsc.ca when you are on your way to the GSC. Once you get to our building, please call our office directly at 604-707-5900 ext. 675411. We will meet you at the lobby to take delivery of the samples.



Technology platform

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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