CANADA'S MICHAEL SMITH
GENOME SCIENCES CENTRE

From Nobel roots.

CONSTRUCTED LIBRARIES

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 is for the submission of sequencing of libraries that were constructed in your laboratory.

Library construction kits should be compatible with standard sequencing primers (i.e. Illumina TruSeq or Nextera primers). If custom sequencing primers or a custom sequencing recipe are required, please contact us prior to submission at *SOW@bcgsc.ca*.

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. LIBRARY CONSTRUCTION

1.1. ADAPTERS

Constructed libraries need to be compatible with standard Illumina sequencing primers (i.e. TruSeq or Nextera) and must be constructed with dual indexed adapters to allow for pooling with other samples. Submission of single index libraries will only be accepted if your project is requesting full lanes of sequencing.

Illumina adapter and primer sequences can be found on the Illumina website. The use of Unique Dual Indexes (UDIs) is strongly recommended to reduce the effect of index hopping.

Libraries constructed using non-TruSeq or Nextera chemistry/adapters must be assessed for compatibility with sequencing requirements.

1.2. BASE DISTRIBUTION BIAS

If your construction method results in libraries with biased base distribution (i.e., an imbalance in the distribution of the 4 bases at any cycle(s), which is common in some library types such as amplicon and certain 10x Genomics libraries, please let us know via the comments section.

Please also confirm if you have any guidance on the appropriate percentage of PhiX genome spike-in to overcome this bias in sequencing. Illumina sequencing instruments can be adversely affected by bias in libraries, potentially affecting base quality and read yield.

1.3. CUSTOM LIBRARY CONSTRUCTION

Please contact us if you are using a custom library construction prior to submission. Primer sequences and index sequences (if applicable) must be provided to the GSC prior to sample submission. We encourage you to perform the research and assess your project's compatibility with our platform.

2. INDEXED LIBRARIES

Index sequences must be provided at the time of submission and should have a balanced mix of bases at each position to ensure no focusing issues arise during the index read(s).

Data from libraries will be split using the provided index sequences. Splitting by index is performed with a one-base-mismatch tolerance (i.e., index reads with one mismatch from the expected index will still be counted towards that index). Therefore, please ensure that no two indices in the same pool are different by less than two bases, as any pair of indices that differ by only one base (e.g. **AGTCCAGT** and **ATTCCAGT**) will be considered ambiguous in the splitting process and reads with either index will be lost from the split bam files.

It is important to ensure that when you submit pooled libraries the indices in each pool contain an equal representation of each base in each position (e.g. do not have all your indices in one pool start with **A**). The instruments do not focus well when all clusters have the same base in the same position.

At the GSC, we ask that all indices be provided to us in the forward format, regardless of the sequencing platform. We will automatically reverse complement the i5 indices as needed depending on the sequencer workflow (forward or reverse). Please refer to Appendix A for details regarding index sequence entry.

Certain sequencing instruments (those with 2-colour chemistry) have a minor restriction on index sequences that can be used when barcoding libraries. To detect a cluster during template generation, there must be at least one base other than **G** in the first five cycles. Index sequences and protocols used for library construction must be confirmed to be compatible with the GSC's sequencing pipeline prior to sample submission.

De-multiplexing at the GSC is optimized for libraries with dual index reads read separately from the insert reads. We strongly recommend against inline indices that are read as part of the insert as this can confound our ability to accurately split reads in complex library pools having potentially large numbers of unassigned reads. If your libraries are constructed using inline indices please contact us prior to submission.

3. LIBRARY QUANTITY & QUALITY

We require that libraries have been purified using a suitable PCR clean-up kit and have an A260/280 ratio of >1.8 and an A260/230 ratio of >1.2.

3.1. QUANTITY CHECK

Once constructed libraries have been approved and submitted, the GSC will assess the quantity of the libraries. If the libraries do not pass the GSC quantity check or if there are apparent issues with the libraries, we will contact you. However, despite passing quantity checks, libraries may not result in satisfactory data.

3.2. QUALITY CHECK

The GSC is not responsible for the quality of submitted constructed libraries or for the generation of data from such libraries. If an additional level of quality check is needed using fragment analysis (e.g. Agilent Bioanalyzer), we can perform the QC at an extra cost.

The Agilent Bioanalyzer can be used to provide visual examination of the constructed libraries. The "perfect" library profile (Figure 1) shows a single peak of the expected size. Common additional forms include primer or adapter dimers and broader bands of lower molecular weight (MW) than the expected peak (Figure 2).

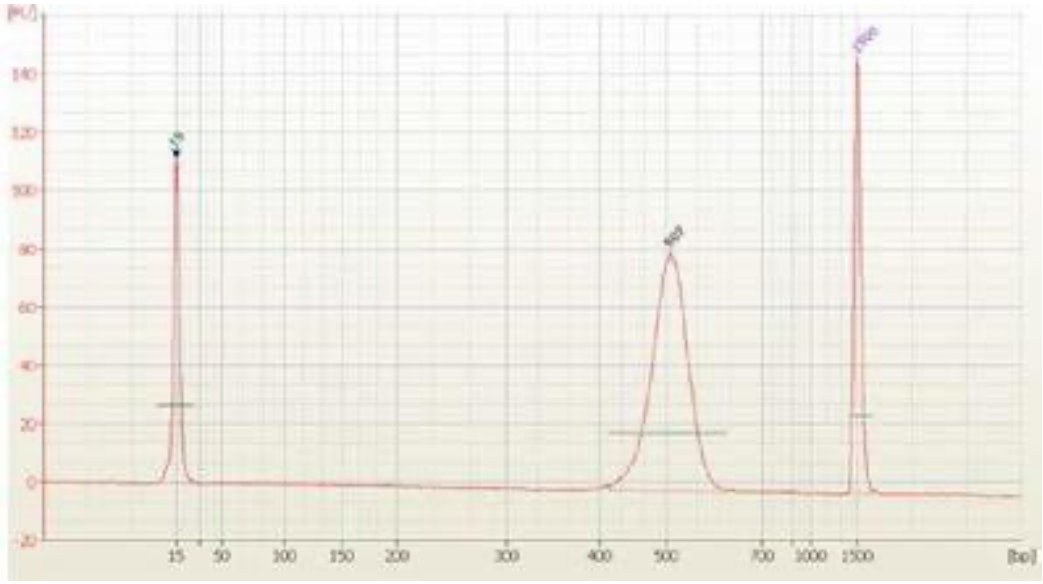


Figure 1. Agilent Bioanalyzer profile of a constructed library.

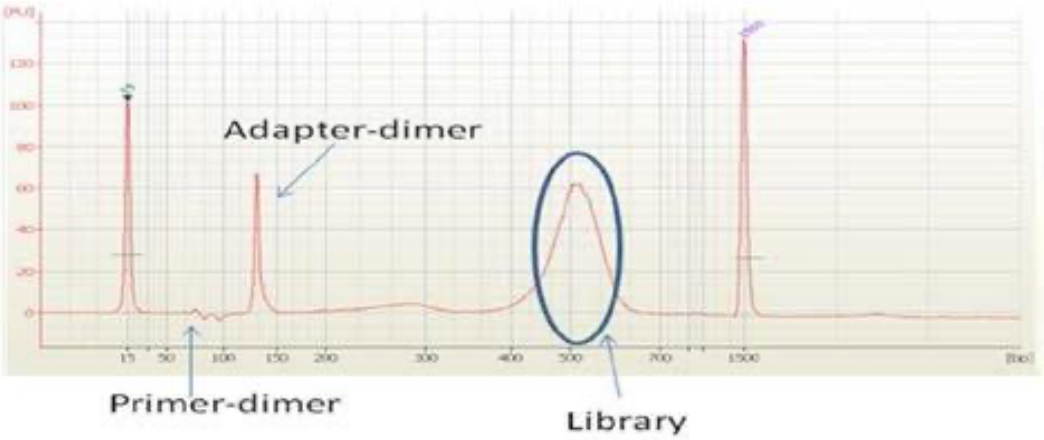


Figure 2. Agilent Bioanalyzer profile showing primer dimers and adapter dimers.

4. LIBRARY TROUBLESHOOTING

The GSC cannot guarantee that constructed libraries will be acceptable for sequencing even after samples have been submitted, and we cannot guarantee the quality of sequencing data from these libraries.

4.1. PRIMER DIMERS

Primer dimers can be minimized by size-selection (e.g. using magnetic beads or gel excision) but may not pose a problem unless they completely dominate the reaction. Useful data has been obtained from libraries despite the presence of 50% primer dimers.

4.2. ADAPTER DIMERS

Sequences of adapter dimers can be overrepresented in the final data files because adapter dimers generate clusters on the flow cell (and therefore sequence) very efficiently. Adapter dimers can be minimized by adjusting the adapter-to-insert ratios during library construction and exercising care in gel extraction or other size selection steps.

4.3. LARGE MOLECULAR WEIGHT

Larger molecular weight fractions are typically broader shaped forms when visualized on the Agilent Bioanalyzer and are probably a result of excess amplification during the final PCR step. While some amounts of larger molecular weight fractions are tolerable, the library should be re-amplified from the gel extracted material if they are too prominent.

4.4. INSERT SIZE

The proper size of the inserts will depend on the application and the type of sequencing performed. As a general guideline, libraries submitted for short-read sequencing should have inserts **no longer than 600 bp**.

5. SUBMISSION REQUIREMENTS

All samples must be submitted in **1.5 mL Eppendorf tubes**.

For maximum yields we strongly recommend that libraries are quantified using **qPCR** or **Qubit**. **Nanodrop** is not recommended because readings are not accurate at the very dilute concentrations utilized in next generation sequencing protocols. Additionally, spectrophotometric methods can overestimate library quantities by including unadaptered (or incorrectly adaptered) products.

Average library size should be determined with the **Agilent Bioanalyzer 2100 High-Sensitivity DNA kit**.

The quantity and concentration of your library is critical. If your sample concentrations/volumes fall just below the minimum required amount, please let us know as we may still be able to run your samples if you do not require maximum sequence yields.

5.1. NOVASEQ X SEQUENCING

For **NovaSeq X** sequencing, we request a minimum of **15 µL** of **4 nM** library resuspended in EB buffer or equivalent. This includes a library volume required for **MiSeq** index quality control before we pool with other in-house libraries. More library may be required for a large number of reads (>600 million).

For a full **NovaSeq X 25B lane** (6 billion reads), **25 µL** of **4 nM** library is required.

For a full **NovaSeq X 25B flow cell** (8 lanes, 48 billion reads), **130 µL** of **4 nM** library is required.

If less than a full lane is requested then external libraries will need to pass quality control prior to pooling for a **NovaX** run, this will be performed by sequencing the libraries at lower depth on a **MiSeq** to confirm indices, bias and cluster density.

5.2. NEXTSEQ 2000 SEQUENCING

For **NextSeq 2000** sequencing, we request a minimum of **21 µL** of **2.5 nM** library resuspended in EB buffer of equivalent.

6. 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.

When you are entering information about the library in our Online Sample Submission system, please pay particular attention to the sections about **library type**, **pool type**, **chemistry version** and **index sequences**. Wrong or missing information in these columns will delay your submission.

6.1. SPECIFYING LIBRARY TYPE, POOL TYPE, AND CHEMISTRY VERSION

1. *Library Type:* Select the type of constructed library you are submitting from the drop-down menu.
    1.1. For **Amplicon** and **Specific Capture**, please add **PhiX recommendations** in the **Comments** section.
2. *Pool Type:* Select from the drop-down menu (e.g., **Illumina Compatible** or **Custom**).
    2.1. Select **Illumina Compatible** if using a library construction kit resulting in libraries that can be sequenced with standard Illumina sequencing primers.
    2.2. Select **Custom** if using a library construction kit that requires any custom sequencing primers. Additional information such as sequencing primer sequences can be provided later in the **Comments** section.
3. *Chemistry Version:* For **Chromium libraries**, select from the drop-down menu (e.g., **V4**). Choose the version based on the **major version**. For example, if your version is **V1.1**, select **V1**.

6.2. SPECIFYING INDEX SEQUENCES

When you are entering information about the library in our Online Sample Submission system, please pay particular attention to the sections about index sequences. Wrong or missing information in these columns will delay your submission.

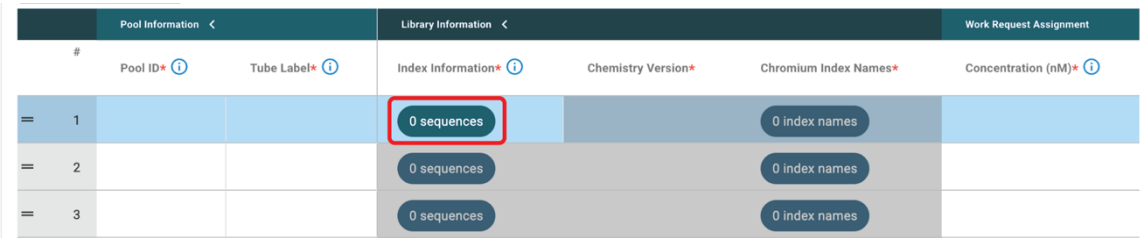
1. *Index Read Type:* Select the expected index primer sequence reads from the drop-down menu (e.g., **Single Index (i7)**). *Your libraries must be constructed with dual indexed adapters to allow for pooling with other samples*.
2. *Contains UMI Adapter?:* Select **Yes**/**No** from the drop-down menu depending whether your pool contains a **UMI Adapter Sequence**.
3. *UMI Adapter Details:* Provide details about the adapter, such as the length of the UMI itself, length of the UMI adapter that needs to be trimmed off, and the location of the UMI (beginning of read 1, index read 2 of the dual index, etc.).
4. *Index Override Cycles:* Enter the override cycles value to indicate the UMI location. Follow Illumina documentation to determine the value for Override Cycles.

5. *Index Information:* Provide the expected index sequence(s). For dual index samples, separate the first index (i7) and the second index (i5) with a hyphen (-) (e.g, **ACGTCATC-TCAGCTAG**). Please see Appendix A (Entering Illumina Nextera Codes on Sample Submission Forms) in this guide.

   5.1. i5 indices must be entered in the forward direction, regardless of which platform will be used for sequencing.

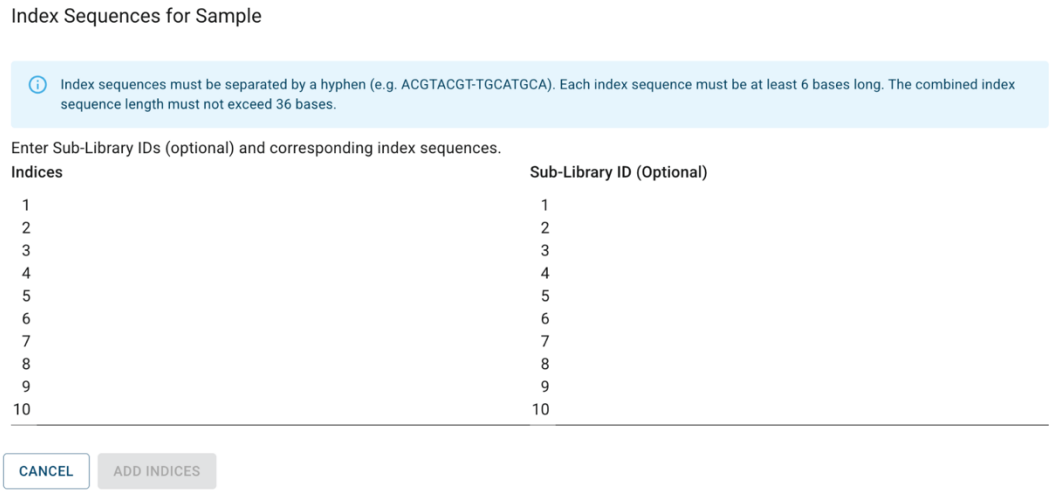
   5.2. To enter Index Information:

      5.2.1. Double-click on the blue 0 sequences button.



      5.2.2. Index Sequences for Sample window will pop up. You can copy and paste directly into the window. Index sequences should be 8 bases or longer unless the submission is for full lanes of sequencing.

      5.2.3. If you are adding a large number of index sequences, please refer to Appendix B (Entering Large Number of Index Sequences on Sample Submission Forms) in this guide.



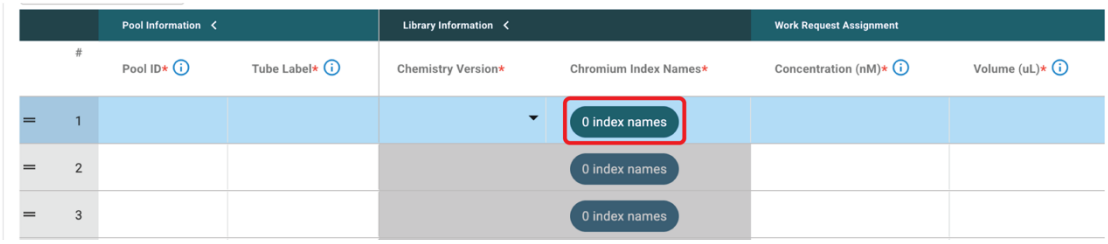
   5.3. Once Indices and Sub-Library ID (optional) have been entered click Add Indices.

   5.4. Make sure the blue button displays the correct number index sequences in your pool.

6. *Chromium Index Names:* For **Chromium libraries**, **enter Chromium Index Names** (such as **SI-GA-A1**) and number of cells for each **Sub-Library** (optional). *Do not enter the Chromium Index Sequences in this field.*

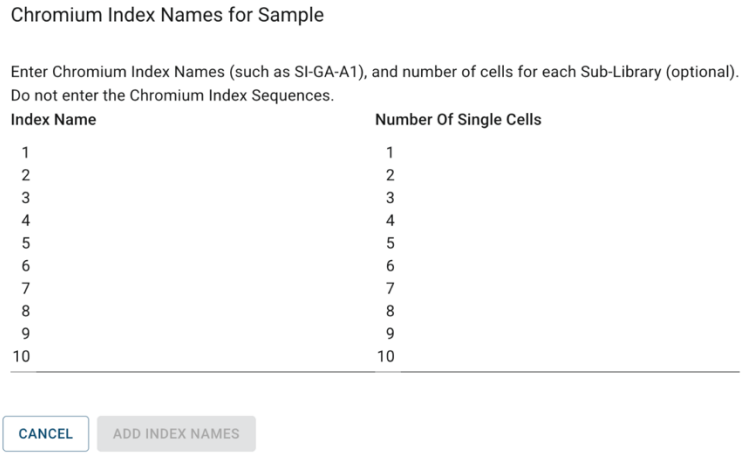
6.1. To enter **Chromium Index Names**:

6.1.1. Double-click on the blue **0 index names** button:



6.1.2. **Chromium Index Names for Sample** will pop-up. You can copy and paste directly into this window:

6.1.3. If you are adding a large number of index sequences, please refer to **Appendix B** (**Entering Large Number of Index Sequences on Sample Submission Forms**) in this guide.



6.1.4. Once **Index Name** and **Number of Single Cells** have been entered, click **Add Index Names**.

6.1.5. Make sure the blue button displays the correct number of index names in your pool.

7. SAMPLE SHIPPING AND DROP-OFF

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

7.1. 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). 

7.2. SAMPLE SUBMISSION BY COURIER

7.2.1. BEFORE SHIPPING

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

7.2.2. 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

7.2.3. 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.

7.3. SAMPLE SUBMISSION BY DROP-OFF

7.3.1. 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.

7.3.2. SAMPLE DROP-OFF AND PLATE PICKUP HOURS

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

7.3.3. 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.

APPENDIX A

ENTERING ILLUMINA NEXTERA CODES ON SAMPLE SUBMISSION FORMS

Documentation provided by Illumina and other manufacturers for dual indexed library construction includes instructions to enter i5 bases differently on sample sheets depending on the sequencer to be used.

The GSC's automated processes require the forward workflow i5 base sequence and will be automatically reverse complemented if an applicable sequencing instrument is used.

Please only provide the NovaSeq 6000 v1.0/MiSeq/NovaSeq X bases.

IDT for Illumina UD Indexes

The IDT for Illumina Unique Dual (UD) index adapters are arranged in the plate to enforce the recommended pairing strategy. The index adapters are 10 bases long, instead of the typical eight bases.

The IDT for Illumina UD Indexes include the following:

- IDT for Illumina DNA/RNA UD Indexes
- IDT for Illumina PCR UD Indexes
- IDT for Illumina Nextera DNA UD Indexes

Index 1 (i7) Adapters

`CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG`

Index 2 (i5) Adapters

`AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC`

▼ Plate A/Set 1 Index Adapters

Refer to *Index 2 (i5) Orientation* for more information on how to enter i5 bases on the sample sheet in forward or reverse complement orientation.

Index Name	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases in Adapter	i5 Bases for Sample Sheet in Forward Orientation	i5 Bases for Sample Sheet in Reverse Complement Orientation
UDP0001	CGCTCAGTTC	GAACTGAGCG	TCGTGGAGCG	TCGTGGAGCG	CGCTCCACGA
UDP0002	TATCTGACCT	AGGTCAGATA	CTACAAGATA	CTACAAGATA	TATCTTGTAG
UDP0003	ATATGAGACG	CGTCTCATAT	TATAGTAGCT	TATAGTAGCT	AGCTACTATA
UDP0004	CTTATGGAAT	ATTCCATAAG	TGCCTGGTGG	TGCCTGGTGG	CCACCAGGCA
UDP0005	TAATCTCGTC	GACGAGATTA	ACATTATCCT	ACATTATCCT	AGGATAATGT

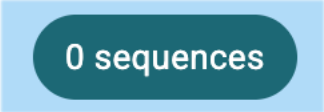
https://support-docs.illumina.com/SHARE/AdapterSequences/Content/IDTIllumina-UDIndexes.htm

APPENDIX B

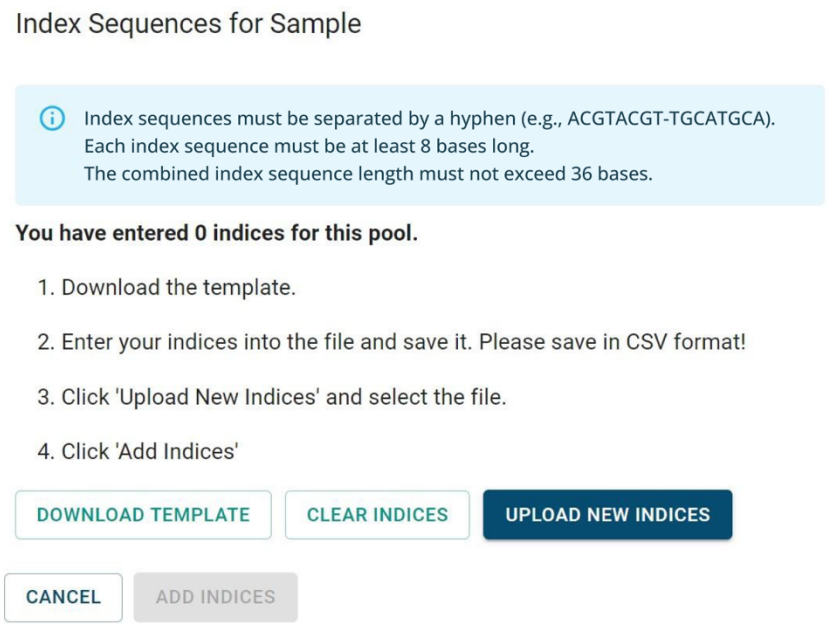
ENTERING LARGE NUMBER OF INDEX SEQUENCES ON SAMPLE SUBMISSION FORMS

To facilitate the entry of the large number of index sequences for single cell whole genome samples, an index entry template can be downloaded.

1. Double-click on the 0 sequences button.



2. A message will pop up with directions and the template for download



3. Follow the directions for index sequence entry making sure to save the file in CSV format. The Sub-Library ID is not mandatory.

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