Novogene

Welcome to the Novogene premade library sample submission guide!

ADVANCING GENOMICS
IMPROVING LIFE

In this guide, you will find a detailed walkthrough of the Novogene sample submission process for premade libraries. Please note that you can reach out to your dedicated technical support representative at any time for help.

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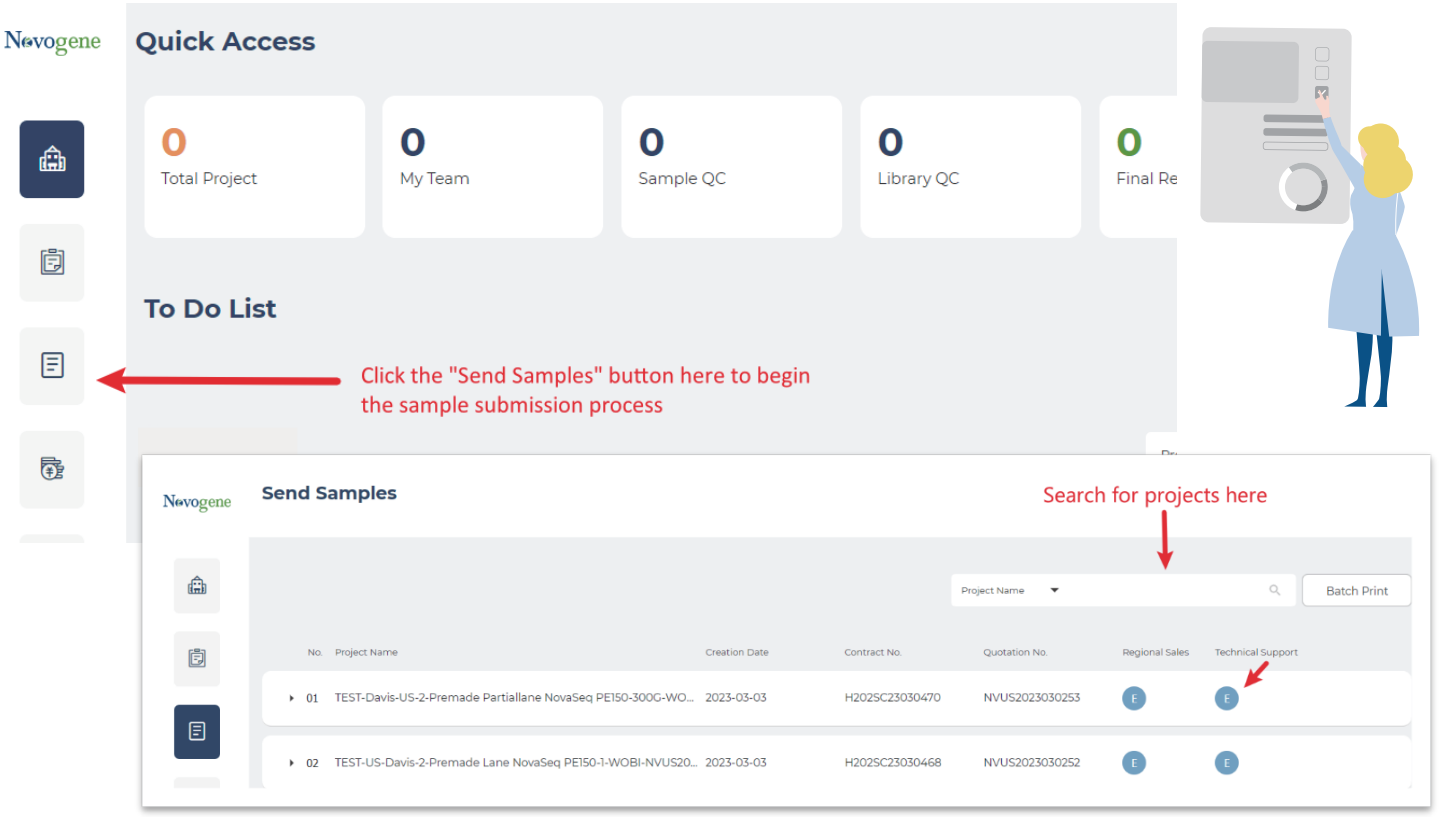
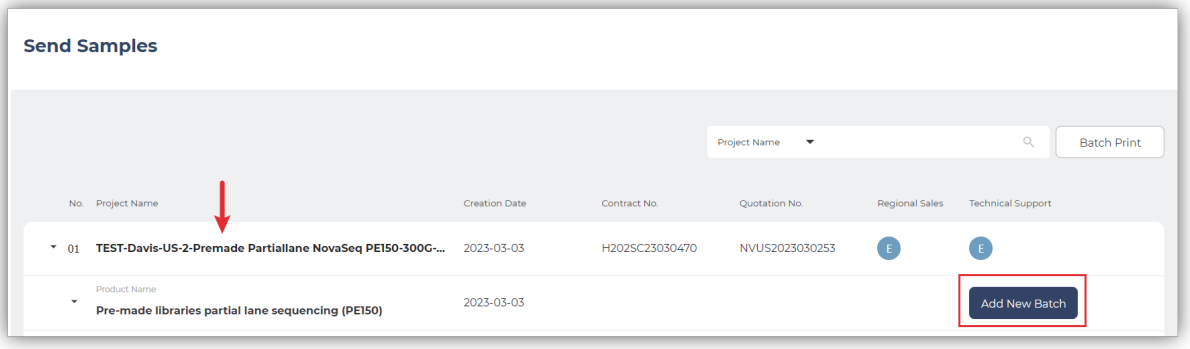
Step 1: Locate your project on CSS



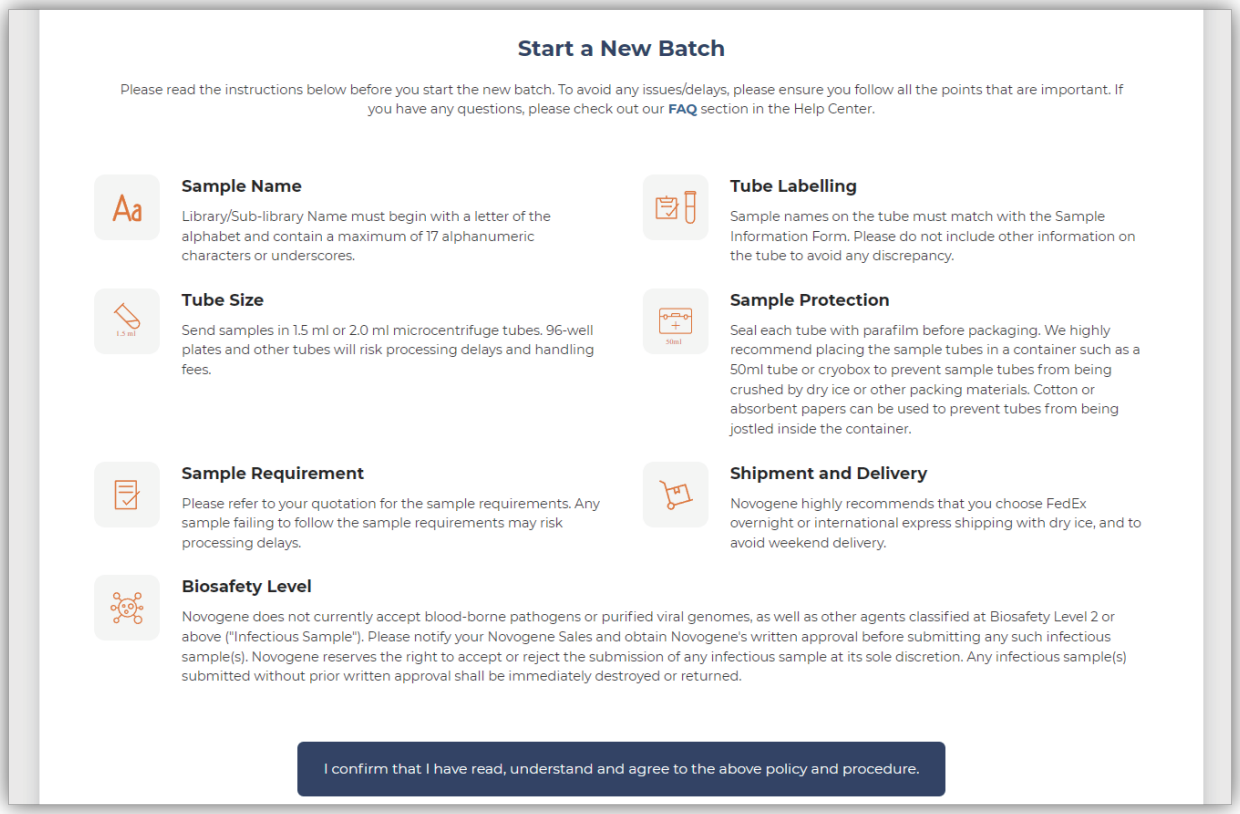
- Here, you'll find all of your Novogene sequencing projects (past and present)
- Your newest project should be at the top of the list
- You can also search for any project using the quotation number in the 'Project Name' search field *(you can find your quotation number on your Novogene quote)*
- Your designated Technical Support representative contact information can be found here too (see image above)

Step 2: Begin a new submission

To begin the sample submission process, click on the project name and then click "**Add New Batch**"



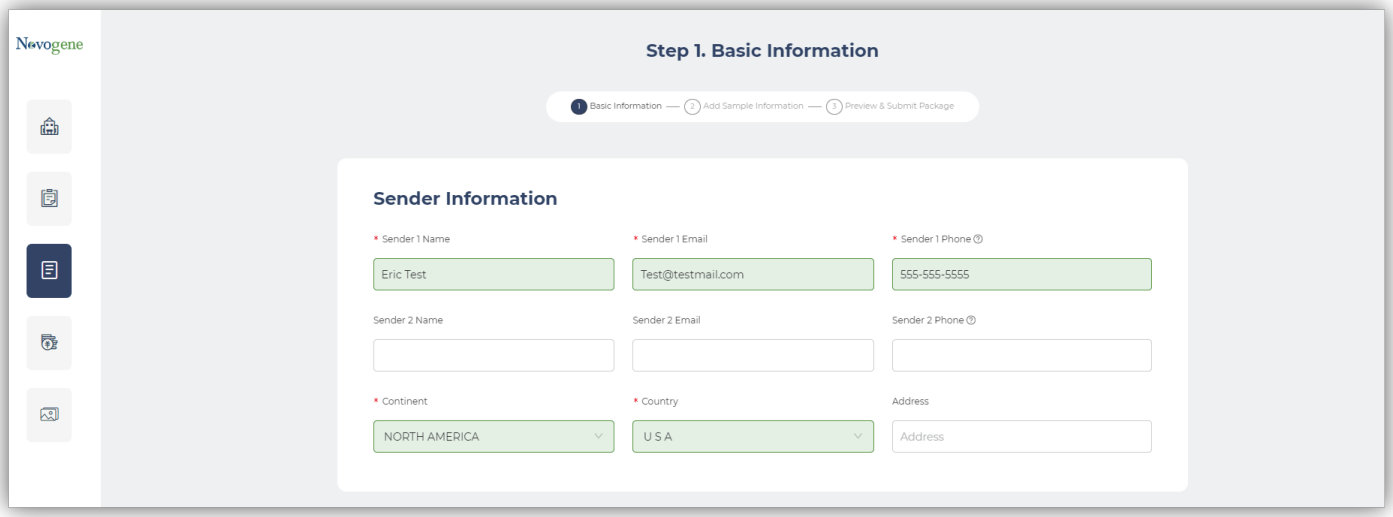
Carefully review all information on the next page. Click **Confirm** to proceed to the next step.



- Following these instructions will ensure your samples are processed without delay
- Novogene sample requirements are listed on your quotation, or they can also be found **HERE**

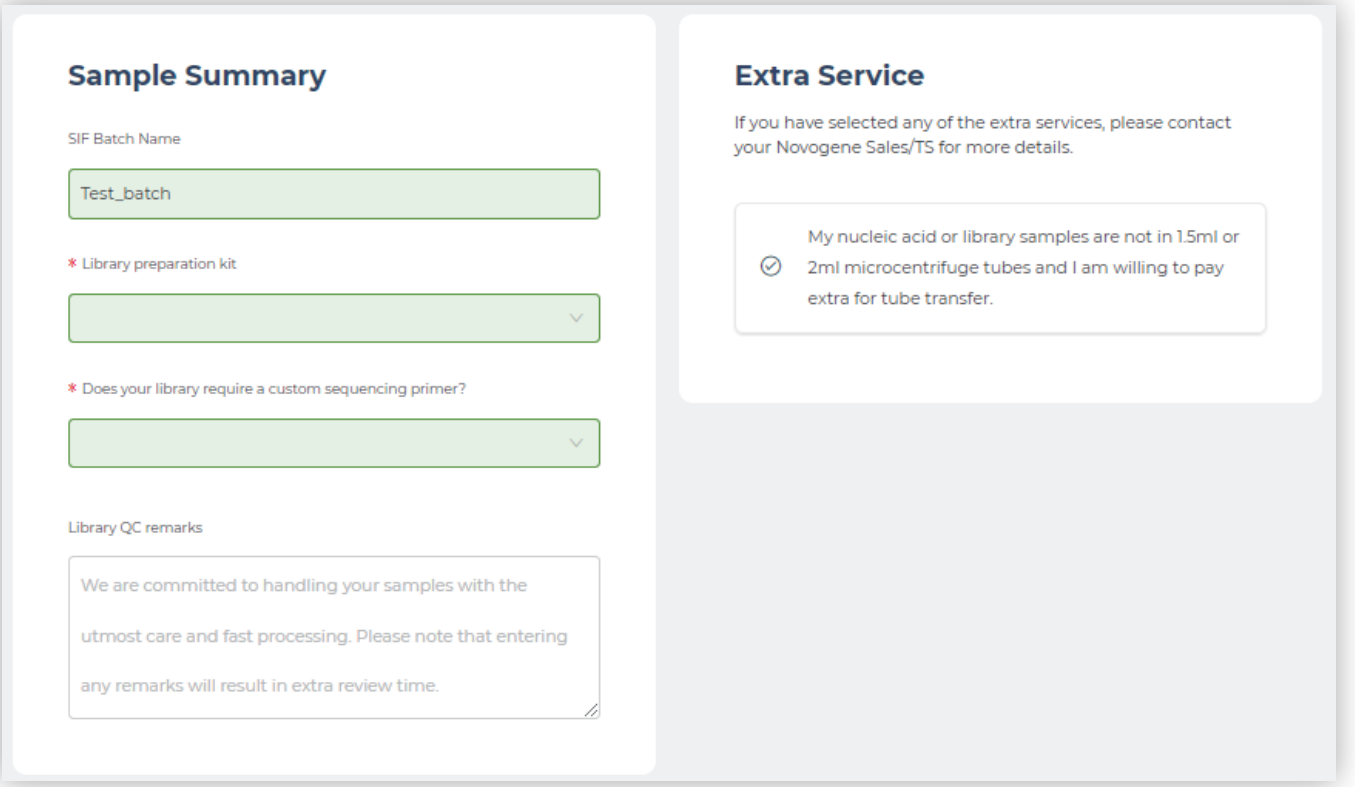
Step 3: Complete basic information

Next, we'll collect some basic information related to your project



- Sender name, email, and phone number should automatically populate (these can also be edited manually)
- If you'd like to add information for a **2nd sender**, you can do so here

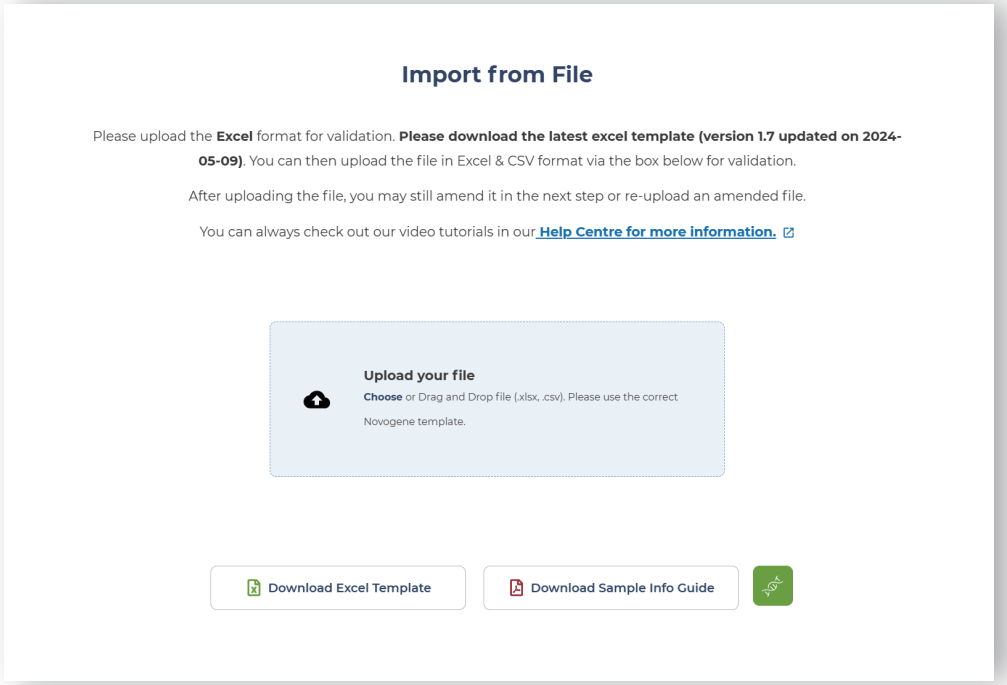
NOTE: the 2nd sender will only receive email confirmation that the sample submission form has been submitted but they will NOT be able to access the project on CSS. To grant additional access to another user, please use the MY TEAM function



- Complete all required fields in the 'Sample Summary' Section (those with an asterisk). When finished, click '**Save & continue**'
- If your project requires a **custom sequencing primer**, please confirm that this was previously communicated to our team *(if you need help with this, or anything else, please contact Technical Support)*

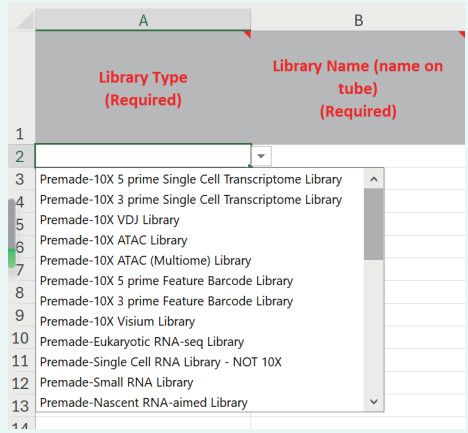
Step 4: Complete the excel template

Next, you will add your sample information to CSS!



- Click **Download Excel Template** to download the sample information excel sheet
- Open the downloaded excel sheet and complete all required fields. See the sections below for more information

Library Type:



- Please choose the correct library type for your sample. See Appendix A for more information.

Tip: For 10X libraries, choose the correct 10X library type to prevent demultiplexing errors. If you do not see your specific 10X library type listed, please check the table in **Appendix A** or contact your Technical Support Representative for help.

Library name and sub-library name:

A	B	C
Library Type (Required)	Library Name (name on tube) (Required)	Sub-library Name(for pooled library) (Required. If is not pooled library, just keep it the same as Library Name)
Premade-Eukaryotic RNA-seq Library	A1	A1
Premade-Eukaryotic RNA-seq Library	A2	SubLib1
Premade-Eukaryotic RNA-seq Library	A3	SubLib2

- Library names are limited to 17 characters and sub-library names are limited to 24 characters. Names must be alphanumeric and cannot contain spaces or any other special characters (#, +, -, etc.). Underscores are allowed but names cannot end in an underscore. Names cannot begin with a number.

  Tip: If you have already labeled your samples, and they violate our naming rules, please contact Technical Support for help

- *Non-Pooled Libraries* (samples containing a single library) – See sample 'A1' above:

  Library Name = Name on the sample tube (must match exactly, no exceptions)

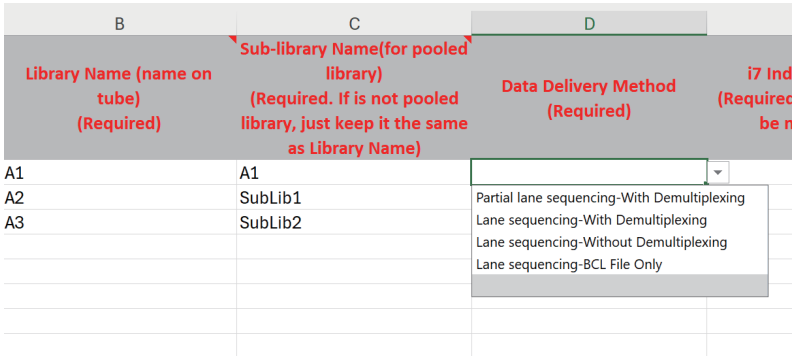
  Sub-library Name = Same as the 'Library Name'

- *Pooled Libraries* (Sample containing multiple libraries, aka sub-libraries, within the same tube) – See sample 'A2' above:

  Library Name = Name on the sample tube (must match exactly, no exceptions). Each sub-library must have the same 'Library Name' (this links all sub-libraries to the same tube/sample)

  Sub-library Name = Must be unique and cannot match any other sub-library name (EXCEPTION: 10X ATAC and 10X ATAC Multiome libraries are composed of four index sequences per sample. For these libraries, please make sure that the sub-library name for each sample is the same.)

Data Delivery Method:



- Choose the data delivery method that meets your project needs:

Lane sequencing (for clients purchasing an entire lane – Novaseq 6000, Novaseq X Plus, or Hiseq)

- *With demultiplexing* → Novogene will demultiplex your data. Index information is required (next section)
- *Without demultiplexing* → Novogene will not demultiplex your data. No index information is required but please inform technical support of the index length if you have not done so already.
- *BCL File* → Delivery of the run BCL file. Index information is not required (please reach out to your Technical Support representative **asap** if you have not done so already)

*If PE adapter is selected as library preparation kit, no need to fill in the index sequence.

Partial Lane sequencing (for clients purchasing a partial Novaseq S4 lane):

- *With demultiplexing* → Novogene will demultiplex your data, requires index information
- *Without demultiplexing* → UNAVAILABLE (partial lane libraries **must** be demultiplexed)

i7 and i5 Index Information:

B	C	E	F
Library Name (name on tube) (Required)	Sub-library Name(for pooled library) (Required. If is not pooled library, just keep it the same as Library Name)	i7 Index Sequences (Required when data need be multiplexing)	i5 Index Sequences
A1	A1	ACTGAGGA	GGATCGAT
A2	SubLib1	CAGCAGTA	TATGCAGT
A2	SubLib2	TGCGAAGC	AGCATGCC

- Index orientation

- All index sequences should be provided in the V1.0 orientation (workflow A/Forward)
- See **Appendix B** for more information

- i7 index information is required for every library/sub-library*
- i5 index is optional (include if your library is dual indexed)

Insert Size and Library Status:

G	H
Insert Size(bp) (Required)	Library Status (Required)
300	Others
300	Others
300	Others

- *Insert Size* → This field specifies the length of the insert of your library and should not include the adapters (check your library prep kit for adapter length). This value can be an average or a range (e.g. "400" or "300-500"). Novogene will measure the library size during QC. All sub-libraries in the same pooled sample must have the same insert size.
- *Library Status* → Select 'others' if your sample is dissolved in water or TE buffer. If library is lyophilized, select 'dry powder' (please note that lyophilized samples incur an additional rehydration fee)

Total Data Amount and Data Unit:

B	C	I	J
Library Name (name on tube) (Required)	Sub-library Name(for pooled library) (Required. If is not pooled library, just keep it the same as Library Name)	Total Data Amount (Required)	Data Unit (Required)
A1	A1	100	G raw data
A2	SubLib1	200	G raw data
A2	SubLib2	200	G raw data

- *Total Data Amount* → Amount of data requested per library

For **full lane sequencing**, the sum of the data amount for all libraries should be equal to the theoretical lane output for a full lane (see below for data amounts and platforms). For **partial lane sequencing**, the sum should add to the quoted data amount.

- NovaSeq 6000 S4 theoretically yields 800G data per lane. A flow cell has 4 lanes, 3200G in total.
- NovaSeq X Plus 10B FC theoretically yields 375G data per lane. A flow cell has 8 lanes, 3000G in total.
- NovaSeq X Plus 25B FC theoretically yields 1000G data per lane. A flow cell has 8 lanes, 8000G in total.

Note about pooled libraries: The data amount listed for each sub-library in the pool should be the same and should reflect the amount of data requested **for the entire pooled sample**.

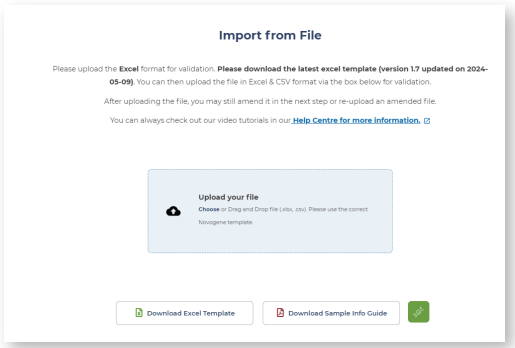
In the example above, the pooled library A2 will receive 200 Gb raw data total. Each sub-library will receive an amount of data that is dependent on the ratio of libraries within the pool (the pooling ratio is determined prior to sending the sample to Novogene and *cannot be adjusted by our lab*)

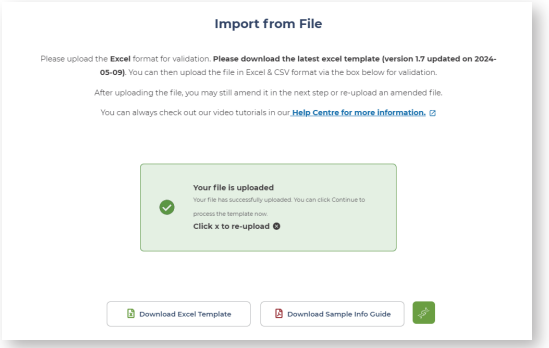
- *Data Unit* → Must be listed as 'G raw data'

If you need assistance converting from 'M raw reads' to 'G raw data', please see **Appendix C** or reach out to your technical support representative for assistance

Step 5: Upload the excel template

Drag and drop the file OR click the '**upload your file**' button and upload the file from your computer

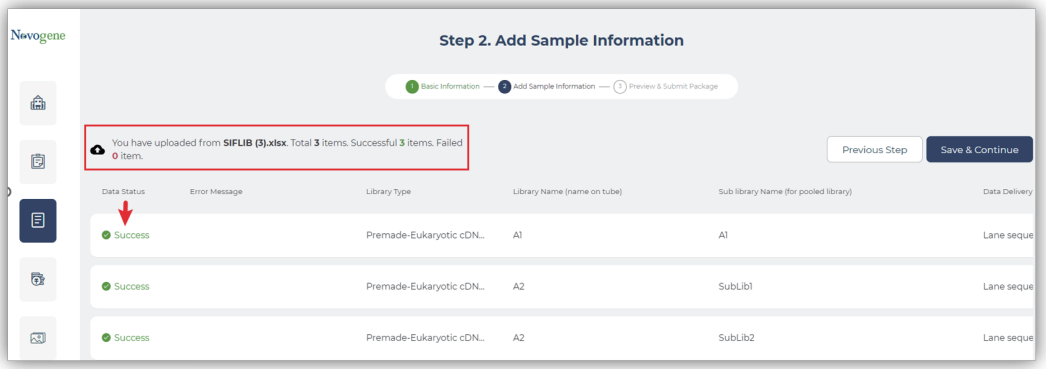




- Once the upload is complete, the upload box should turn from **blue** to **green**
- Click '**Continue**'

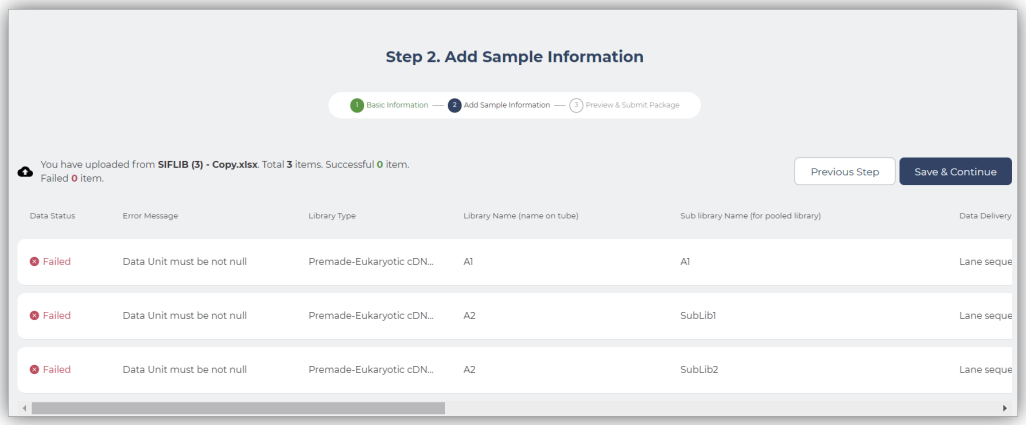
Step 6: Confirm all samples were uploaded successfully

After uploading the excel file, the system will review the information and will notify you of any issues



- At the top of the screen, you can view the number of **successful** and **failed** samples
  In this example, all three libraries were uploaded successfully (no issues)
- Click **Save & Continue** if the file was uploaded correctly. If not, see the example below for further instructions.

Example: One or more samples failed to upload



- Above is an example of a failed upload
- A description of the problem will appear under the 'Error Message' field (in this example, the data unit was not specified, and the upload failed)

To revise:

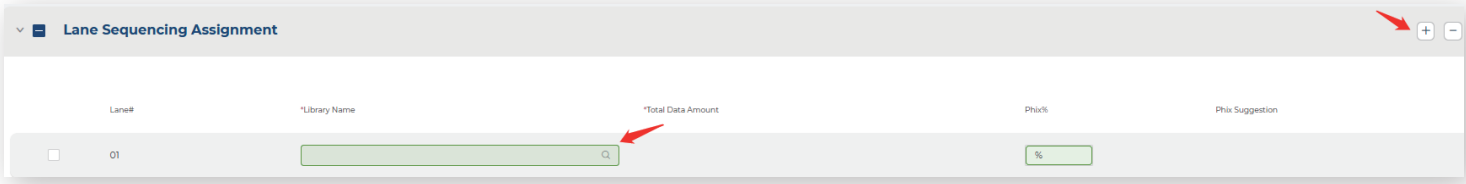
1.Click **Previous Step**
2.Fix the mistake on the excel form
3.Re-upload the edited/fixed form and click **Continue**
4.Make sure all samples are listed as successful. If so, click **Save & Continue**

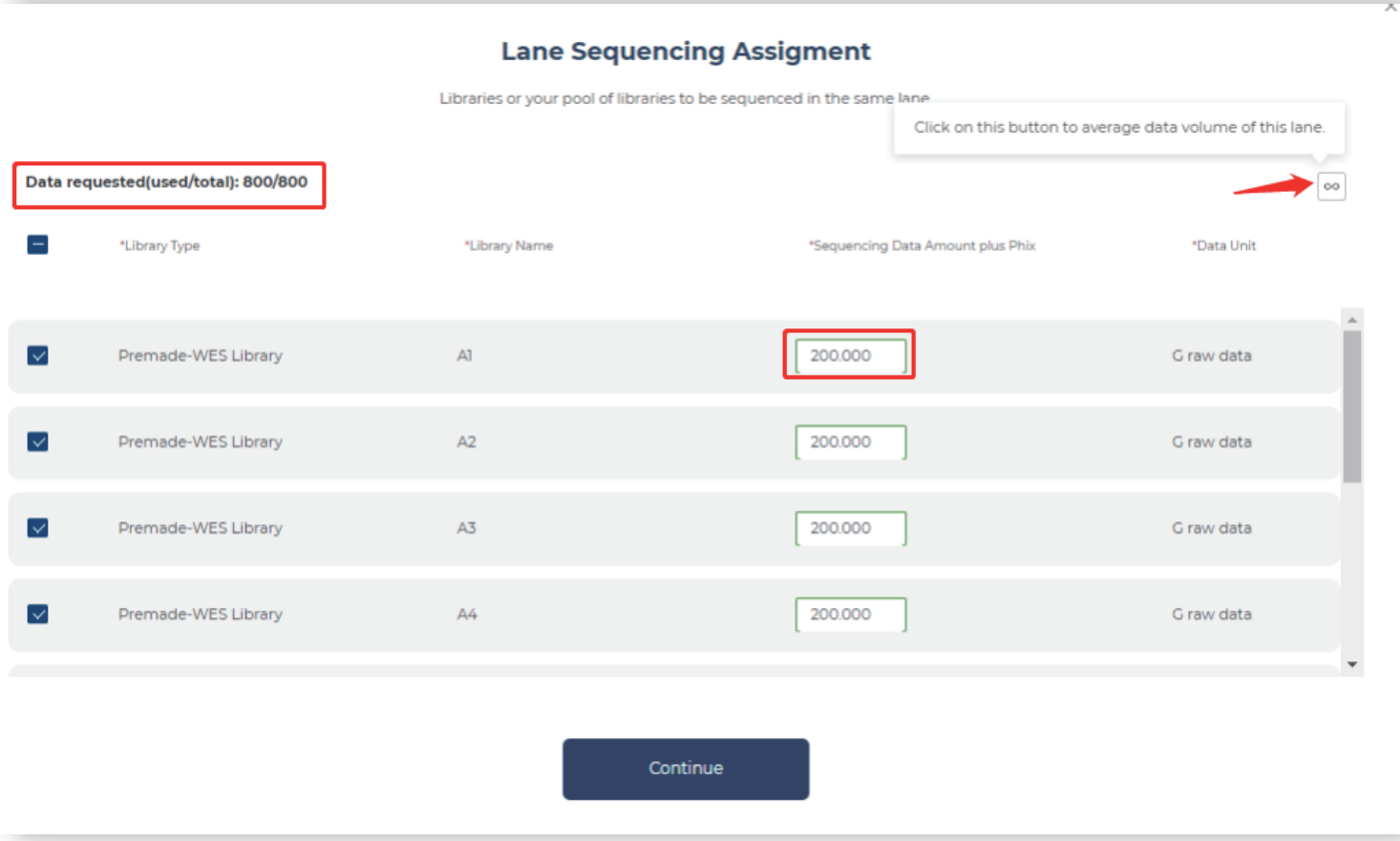
Step 7: Review and lane assignment*

Lane assignment is only for full lane sequencing projects.* ***Partial lane customers please skip to the section below*

1. Scroll down to the '**Lane Sequencing Assignment**' section
2. Click the '+' symbol to add a new lane. Then, in the "**Library Name**" field, click on the magnifying glass icon to open a new window (*see 1st image below*)
3. Select the libraries to be sequenced in this lane. Confirm the sum of the data is equal to the theoretical lane output (*See 2nd image below. This is an example of an S4 lane with a theoretical output of 800G*)
   - NovaSeq 6000 S4 theoretically yields **800G** data per lane
   - NovaSeq X Plus 10B theoretically yields **375G** per lane
   - NovaSeq X Plus 25B theoretically yields **1000G** data per lane

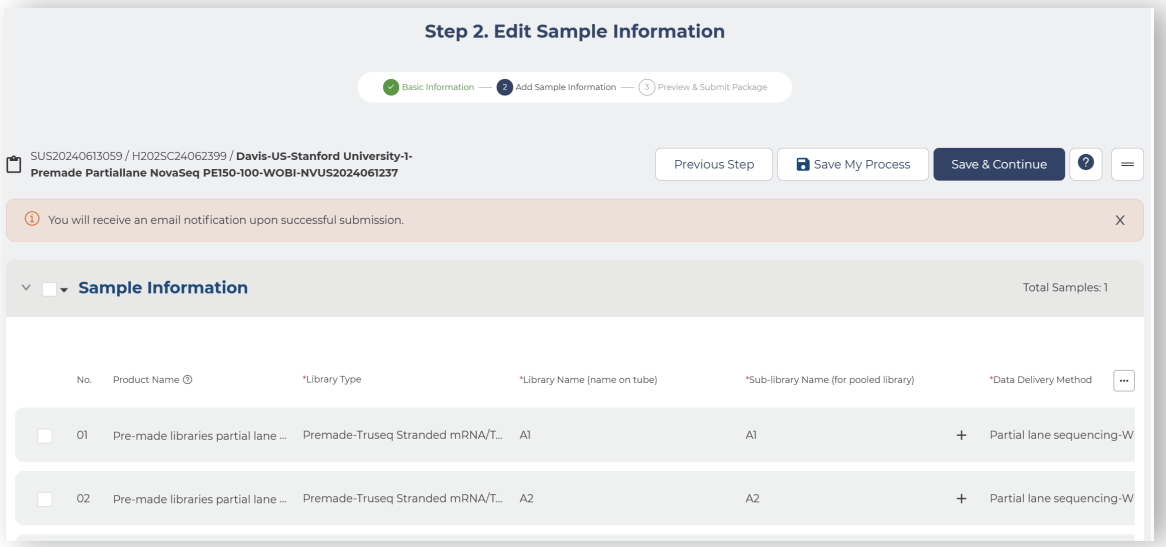
4. Note that the ∞ **symbol** can be used to **average the data amount per library** across the lane (i.e. all libraries will have the same data output if this button is clicked). The data amount per library can also be manually adjusted.
5. When ready, click '**continue**'
6. Adjust the % of Phix spike-in per lane.
   - If no PhiX is required, leave this field blank (do NOT add '0%')
   - If you are not sure about the % of PhiX, please check your library prep kit or reach out to Technical Support
   - **Note**: PhiX will take up space on the lane and will reduce the amount of data available for your libraries. If 10% PhiX is spiked-in, this will reduce the amount of data available to your libraries by 10%. (*For example*: if 10% PhiX is spiked into the S4 lane below, we will see ~80G of data attributed to PhiX. These libraries would not produce 200G of data but would instead produce ~180G or 10% less due to the PhiX spike-in)
7. Click '**save and continue**' in the top right of the screen





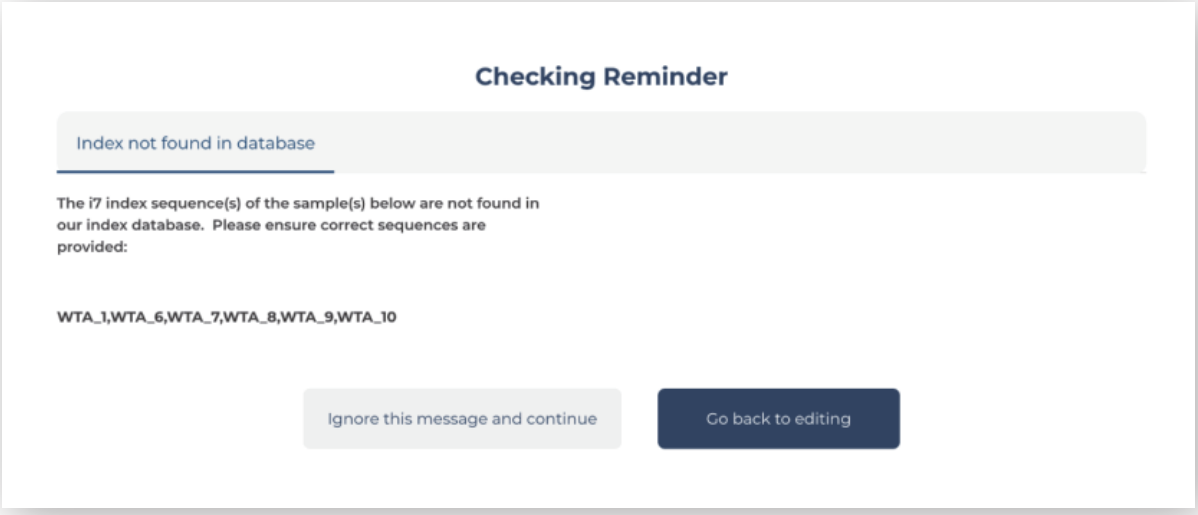
Partial lane customers:

- Review all information one more time, then click '**Save & Continue**' if everything is correct



Step 8: Index check

All index sequences are compared against a database to help ensure the sequences are in the correct orientation for demultiplexing. You may be prompted to review your index sequences if any potential issues are detected



- Error code: *Index not found in database*

- Libraries with an index that is not listed in the database. This could be due to a mistake in the sequence or because the index is part of a kit that is not included in the database*

• Error code: *Possible reverse complements*

• Libraries with an index *potentially** submitted in the incorrect orientation will be listed here
• **Reminder**: all indexes should be submitted using the V1.0 orientation (workflow A/Forward)

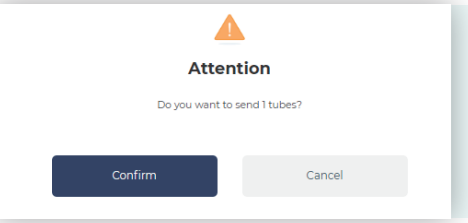
* Please note that our database does not include ALL index sequences for every kit/manufacturer. If you are confident your index is in the correct orientation, you can ignore these warnings and proceed. ***Keep in mind that additional fees/penalties may apply if the incorrect index results in complications during demultiplexing.***

To Continue → Click '**Ignore this message and continue**'

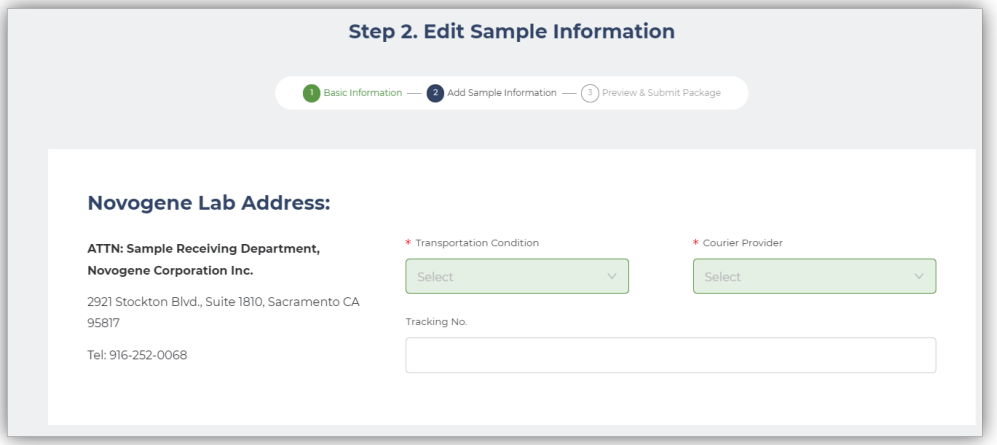
To Revise → Click '**Go back to editing**' and complete the following steps:

❶ Click '**Previous Step**'
❷ Edit/revise the index sequences on your excel form and re-upload when finished
❸ Proceed through all other steps, as indicated above, then click '**Save & Continue**' to proceed to the final steps

A pop-up window showing tube number will appear right after index check to confirm if all samples information is successfully uploaded.



Step 9: Provide shipping information and review the pre-shipping checklist



• Select the correct transportation condition from the dropdown list

• We highly recommend that you include dry ice in all shipments.
• How much dry ice should I include? At least 10 lb per day of shipping (e.g. overnight shipments should include ~10 lb of dry ice)
• Can I ship samples on any day of the week? No, please only ship samples Mon – Wed to help minimize shipping-related issues/delays

- Select the shipping courier from the dropdown list
- Provide a Tracking #, if available (if not, no worries! Please share the tracking # with your Technical Support representative when you have it)
- **Novogene Shipping Address:**

ATTN: Sample Receiving Department
Novogene Corporation Inc.
2921 Stockton Blvd.
Suite 1810
Sacramento, CA 95817
Tel: 916-252-0068

Please take a moment to review all shipping instructions (*this can prevent any unnecessary delays and will ensure your samples are processed efficiently):*

Samples classified as Biosafety **Level 2 (BSL-2)** or above ("infectious samples") will not be accepted unless they have a written approval from Novogene. If you have samples that are classified above BSL-2, please contact your sales manager for more details.

Samples are in 1.5 or 2.0ml flip cap Eppendorf tubes, and are labelled in black permanent marker at the top and side of each tube matching the sample name submitted. Samples are properly sealed with parafilm and cushioned enough to avoid damage. OR Samples are in the appropriate tube/slide/other formats required for extraction and are labelled in black permanent marker accordingly. OR Samples are not in 1.5 or 2.0ml flip cap Eppendorf tubes, and I am aware that additional charges may apply.	No
Samples are packed with appropriate shipping conditions such as ambient temperature, gel pack or dry ice. For dry ice, we recommend packing in 10 lb/5 kg for overnight shipping or two-day shipping depending on your local shipment options.	No
Shipment will be delivered on weekdays. We recommend shipping with overnight or international express.	No
Samples do not contain any potential or known infectious material such as pathogens, infectious genetic materials etc. OR Prior written approval from Novogene was obtained for samples that may contain any potential or known infectious material.	No
Please put the hard copy of the Sample Information Form (SIF) in the parcel.	No

- After reviewing each item, click the toggle to **YES**
- Please remember that **we cannot accept** any samples that are classified as **BSL2 or above**. If you are sending samples BSL2 or higher (or you are not sure), *please contact your Technical Support representative before shipping*

Step 10: Final review and submission

- In this final step, please take one final opportunity to review your sample information
- If everything looks correct, click **Submit**
- **Congratulations**, you're finished! And you will receive an email notification confirming your submission.

Step 11: Technical support review and approval

What happens after you submit the Sample Information Form?

1. Your designated Technical Support representative will review your submission within 1-2 hr
2. After reviewing, your Technical Support representative will either approve the form (if everything looks good), or reject the form (if there is a problem)

   If Approved → You'll receive an email notification confirming that you can ship your samples.

   If Rejected → You'll receive an email notifying you that the form was rejected. Your Technical Support representative will reach out with more information to help you correct the form. After making those corrections, you'll need to re-submit the form (following the steps above).

Thank you for taking the time to review our sample submission guide. Please don't hesitate to contact us if you have ANY questions. Happy sequencing!

Appendix A: Choosing the correct 10X library type on the Sample Information Form

Please use the table below to help select the correct library type for 10X Genomics libraries and other related libraries. If you do not see your specific library type here, please reach out to Technical Support for help

Corresponding 10X Library Type	Library Type on SIF
• 10X scRNA 3' libraries (single and dual index), • 10X scRNA Gene Expression library from 10X Chromium sc Multiome ATAC + Gene Expression Reagent Kits • 10X Fixed RNA Profiling, aka 10X Single Cell Gene Expression Flex (dual index, 10 bp)	Premade-10X 3 prime Single Cell Transcriptome Library
• 10X scRNA 5' libraries (single and dual index)	Premade-10X 5 prime Single Cell Transcriptome Library
• 10X VDJ libraries (single and dual index)	Premade-10X VDJ Library
• 10X scATAC	Premade-10X ATAC Library
• 10X scATAC library from 10X Chromium sc Multiome ATAC + Gene Expression Reagent Kits	Premade-10X ATAC (Multiome) Library
• 10X Cell Surface Protein (CSP), Feature Barcoding for Cell Surface Protein, CRISPR Screen, Cell Multiplexing. Other libraries using 10X library kits - CITE-seq, ADT, Cell Hashing, Hashtag, ASAP-seq	Premade-10X 5 prime Feature Barcode Library or Premade-10X 3 prime Feature Barcode Library
• 10x Visium Spatial Gene Expression	Premade-10X Visium Library

Appendix B: Choosing the correct index orientation

Our demultiplexing software requires the index sequence in V1.0 orientation (workflow A/forward).

For the i7 index, please refer to "i7 bases for sample sheet" in your library preparation guide.

For the i5 index, please select "i5 Bases for Sample Sheet NovaSeq 6000 with v1.0 reagent kits".

Here is an example from Illumina:

Index Name	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases in Adapter	i5 Bases for Sample Sheet NovaSeq 6000 with v1.0 reagent kits, NovaSeq X Series, MiniSeq with Rapid reagents, MiSeq, HiSeq 2000/2500, NextSeq 1000/2000 (Sample Sheet v2)	i5 Bases for Sample Sheet iSeq, NovaSeq 6000 with v1.5 reagent kits, MiniSeq, NextSeq 500/550, HiSeq 3000/4000/X, NextSeq 1000/2000 (Sample Sheet v1)
UDI0001	CCGCGGTT	CCGCGGTT	AGCGCTAG	AGCGCTAG	CTAGCGCT
UDI0002	TTATAACC	TTATAACC	GATATCGA	GATATCGA	TCGATATC
UDI0003	GGACTTGG	GGACTTGG	CGCAGACG	CGCAGACG	CGTCTGCG

Here is an example from 10X Genomics Dual Index Kit TT, Set A:

	A	B	C	D	E
1	# Workflow A = Illumina Forward Strand Sequencing Workflow				
2	# Workflow B = Illumina Reverse Complement Sequencing Workflow				
4	index_nar	index(i7)	index2_workflow_a(i5)	index2_workflow_b(i5)	
5	SI-TT-A1	GTAACAT(	AGTGTTACCT	AGGTAACACT	
6	SI-TT-A2	GTGGATC/	GCCAACCCTG	CAGGGTTGGC	
7	SI-TT-A3	CACTACG/	TTAGACTGAT	ATCAGTCTAA	

Appendix C: Converting from M raw read pairs to G of raw data

Please use the formula below to quickly convert from M raw reads to G. Note that this formula is only applicable for PE150 sequencing projects.

G of raw data = (# of M raw read pairs) x (0.3)

Example:

20 M read pairs = ??? G of raw data

G of raw data = (M read pairs) x (0.3) = (20) x (0.3) = 6 G of raw data

Why is 0.3 used in the equation? This corresponds to 300 bp, which is the combined read length of read 1 and read 2 in PE150 sequencing. We would obtain the same answer if we multiplied 300 bp by 20 million (try it!)

Novogene

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