HTSF FAQs

Frequently Asked Questions

Always feel to reach out to the Customer Service staff at the HTSF if you are unable to find an answer to your questions.

Getting Started

How do we get started?

Read over the detailed instructions on the Submissions Tab first. Then contact the customer service team regarding your planned projects. You will be provided with a Project Initiation document which should be completed before submitting your samples. A meeting with Piotr Mieczkowski, the HTSF Research Technologies Director, may also be required. We will discuss your scientific objectives so we can suggest options that will meet your data goals and conserve funds.

Are the HTSF's services open to researchers that are not affiliated with UNC?

The HTSF is open to all researchers at UNC-CH, other academic institutions, industry and international studies.

- US ACADEMIC RESEARCHERS and INDUSTRY- HTSF customer service staff will assist with the submissions of materials to the TracSeq system. The system is currently only accessible to UNC staff as it is password protected. All researchers outside of UNC-CH will need to provide a copy of a valid purchase order before submissions can be done.
    - The HTSF will provide an official quote for non-UNC researchers to create a PO at their institution.
    - The fees for service will have an F&A fee added as required by UNC Chapel Hill. HTSF does not control the percent F&A we are required to add. UNC annually determines the F&A percent. Please contact us for more information.

- INTERNATIONAL STUDIES – HTSF customer service staff will assist with the submissions of materials to the TracSeq system. The system is currently only accessible to UNC staff as it is password protected. International Researchers are required to prepay for agreed upon work. The HTSF will provide an official invoice and electronic payment instructions for the research to submit to their accounting department.
    - The research needs to HTSF accounting with the check #, name of the bank it was drawn on and date of the check. UNC will confirm the check has been received. At that point, a submission can be completed.
    - The fees for service will have an F&A fee added as required by UNC Chapel Hill. HTSF does not control the percent F&A we are required to add. UNC annually determines the F&A percent. Please contact us for more information.

If I have an account with the HTSF but I am starting a new experiment, do I need a new account?

A new account is not needed for each experiment. An HTSF *Account Name* is a way to organize the people who work with a specific PI, have approval to submit samples, see data related to samples and who has permission to make requests/ processing approval about those samples. It also allows us to organize samples by *Account Name* within our databases. In this manner we can have multiple material types processed in different manners under a single account. Each submission is assigned a unique batch number. The BATCH NUMBER equates to an experiment within your group. The information for the batch is loaded into the processing databases for the HTSF staff to view as they work. And this allows us to have multiple batches for a single account run through processing at the same time.

That being said, you may need to submit a Project Update Form to the customer service staff if you are going to be requesting new service not previously done by your group OR if it has been

a significant time since the account has been active. The Account Update Request Form can be found on *Forms and Guides Tab, General*.

Submitting to the HTSF

How can I submit my samples if I am not a UNC researcher?

Please contact the customer service team for more information on how to proceed. They will assist you with creating an HTSF account if you do not already have one. Additionally, they will provide you with the documents to complete before submitting. For information for External service billing, see above "Are *the HTSF's services open to researchers that are not affiliated with UNC?"*

How should I ship my samples?

If you are unable to physically drop off your samples to the HTSF, you are welcome to ship your samples instead. Detailed information on how to ship your raw material, libraries, or pools can be found in HTSF Shipping and Packing Instructions document under *Forms and Guide tab, General*.

Can I submit any number of samples?

We welcome next-generation sequencing projects of all sizes, including both large and small runs. Unlike larger industrial sequencing facilities, UNC HTSF has no minimum sample size. This makes it cost-effective to perform advanced, higher-risk applications that are often more expensive at other centers.

We also encourage pilot projects: You can *test* your experiment at our facilities by sequencing just a small number of samples for the reasonable price per sample. This flexibility is often particularly useful for researchers who are new to the technology. Or trying to utilize current techniques in a novel manner.

For some services, we offer discounts for large sample groups. We have break point for several of our standard high throughput libraries which are cheaper when submitted in a plate of 96 versus a single group of 8. For sequencing on the HiSeq systems, if you can fill an entire flowcell of 8 lanes, we give you a per lane discount. The discount varies according to the platform and sequencing set up. Please inquire.

How do I drop my samples off at the HTSF and what do I need to bring with me?

When completing drop off, please bring your samples and your delivery manifest to the HTSF. The delivery manifest can be downloaded and printed from your computer after approving your quote from TracSeq. You may then bring your material to 1153 Genome Sciences Building between the hours of 9am to 4pm Monday through Friday. If you are unsure where HTSF is located, see Where is the HTSF? In the *Forms and Guides Tab, General*.

Where and when can I drop off my samples?

Sample drop-offs are from 9am to 4pm (Mon-Fri) at 1153 Genome Sciences Building. Due to UNC security considerations, our facilities may not be accessible outside these hours. Use this link for a detailed guide on how to locate the HTSF. Where is the HTSF? Is found in *Forms and Guides tab, General.*

A COVID updated note: During the COVID19 UNC School of Medicine limited staffing period, HTSF no longer allows studies to drop off materials at will. Once a submission is approved, you will be contacted to arrange a 30min window for drop off. Drop off may not happen until the next weekday, if we are booked. We ask that you be patient for a drop off window. We have to consider the staffing of the lab and other studies coming to drop materials in order to keep the number of people present to our mandated levels. HTSF does have a no contact drop off arranged for use during your window. For those in the GSB, we request you do not drop materials outside of this window. The non contact freezer is not monitored or secured. For **ALL USER** labs, we request that you call the HTSF customer service number once you have dropped off materials. It is posted over the drop off area and on the exterior door.

Can I submit my samples in a 96-well plate?

If you are submitting 24 or more samples, we recommend using a plate instead of tubes. Additionally, we need plates filled and submitted in **columns format** (e.g. A1, B1, C1, ... H1, then A2, B2, C2, ... H2). Do not load plates in row format. This is required to meet the needs of our robots and is what allows us to provide discounts for some library services. See Submission tab for more details on plate submission.

Plates must be BioRad 96 well Skirted PCR plate, hard-shelled (Catalog number: HSP-9631). See the HTSF Approved Container Requirements in the *Forms and Guide tab, General.*

How do I ensure that my DNA is high quality?

Please reference the HTSF DNA and RNA Sample Preparation Requirement Guide on the *Forms and Guides tab, Illumina* for minimum input for different materials for library preparations and pools for sequencing.

- Make sure it is double-stranded.
- Has undergone as little freeze-thaw cycles as possible. HMW DNA it is best to keep the DNA at 4°C. For procedures that required long stranded DNA, please contact the HTSF for the best extraction methods.
- Has not been exposed to high temperatures (>65°C for 1 h can cause a detectable decrease in sequence quality).
- Has not been exposed to pH extremes (<6 or >9).
- Does not contain insoluble material and is not colored or cloudy.
- Does not contain RNA. Always RNAse treat the DNA.
- Has not been exposed to intercalating fluorescent dyes or UV radiation.
- Does not contain chelating agents (e.g., EDTA), divalent metal cations (like Mg 2+), denaturants (like guanidinium salts, phenol), or detergents (like SDS, Triton-X100).
- Does not contain carryover contamination from the starting organism/tissue (like heme, humic acid, polyphenols).

How do I ensure that my RNA is high quality?

Please reference the HTSF DNA and RNA Sample Preparation Requirement Guide on the *Forms and Guides tab, Illumina* for minimum input for different materials for library preparations and pools for sequencing. Additional suggestions can be found on the *Submission tab, Submission Sample Preparation Requirements for Submission.*

- Make sure that your RNA is stored in a -80 freezer
- Has undergone as little freeze-thaw cycles as possible
- Does not contain insoluble material and is not colored or cloudy.
- Does not contain DNA. Always DNAse treat the RNA.
- Use a Bioanalyzer or TapeStation to determine the RIN value.

What eluent /suspension buffer should my sample be in?

Samples may be suspended in different types of buffer, including 10mM Tris, dH2O, or Qiagen EB. However, we prefer when samples are suspended in dH2O. It will allow us to concentrate the samples when necessary without having to worry about concentrating the salts present in other types of buffer. High salt (EDTA) concentration can interfere with the various enzymes that are used during library preparation and prevent sequencing. Do not samples in buffer with EDTA.

I need help with my finding things on TracSeq for my submissions.

The HTSF customer service group can help you with issues. If you are having issues with your submissions, see Submission Tab for detailed help and links to guides. Navigating TracSeq, in the *Forms and Guides, TracSeq and Submissions* are detailed direction on a number of topics

If you have already submitted but need to check something out about your submission, it helps if you know your batch number of interest. But HTSF customer service can help you determine the batch number if needed. There are several items you can find on TracSeq about your submission

- Review a quote
- Print out a confirmed manifest
- Details of your original requests
- Notes on processing of your samples
- QAQC information is attached to each batch
- Run names
- Run Total reads
- Run Demultiplexed reads
- Sequencing Run set up info
    - % PhiX loaded of run
    - Amount pool loaded
    - Ave fragment size of the pool
    - Conc. of the pool
    - Molarity of the pool
- Sequencing Run metrics for each library
    - Clusters
    - % perfect match
    - Q30
- Data folder link for your data

There is a Navigating TracSeq guide that can be found in *Forms and Guides, General*. It has instructions to find all of these details and more.

Timeline

How long will it take to get my results?

Samples are processed on a first come first serve basis. Please reach out to us to see where the status of queues as this may assist you with determining which platform is closer to being filled and therefore has a shorter waiting period. When submitting raw material, you should expect

results in approximately 4 to 6 weeks after QAQCing the raw material and determining that it meets HTSF quality metrics. The actual library preparation is approximately 1 week once it begins. When submitting libraries or pools, your data can be distributed between 3-5 weeks, depending on sequencing platform. Typically, the time lines are much shorter.

If you have a data deadline for a paper, a grant, or a presentation, please contact the customer service team prior to submission. We want to confirm we can meet a desired deadline. We can make suggestion on how to make processing move faster if you are flexible.

This estimate of delivery can vary based on the selected sequencing platform. The following platforms will take more time to fill due to low demand:

- HiSeq 2500 all set ups, really only used for legacy studies
- HiSeq 4000 single end 50x
- HiSeq 4000 paired end 150x

THESE PLATFORMS WILL BE DISCONTNUED BY THE END OF 2022 (or sooner) **BECAUSE ILLUMNA NO LONGER FULLY SUPPORTS THES SYSTEMS**

The Miseq, Nextseq and Novaseq platforms run frequently. Timeline for sequencing is based on availability of the machine once your sample or pool is ready. MISEQ/ Miseq nano/ Nextseq are single lane systems.

Novaseq SP/ S1/ S2 are 2 lanes systems which the HTSF requires a study to fill exclusively. The HTSF can run separate samples on each lane by using XP mode loading. There is an extra fee for this service.

The Novaseq S4 (10B cluster system) is 4 lanes. We can run this either with a single pool from 1 study (standard loading mode). Or with a different pool from a single or series of studies. It will be run in XP mode for loading. If a study wants less than 4 lanes for their pool, HTSF will accept any number of lanes and complete the flowcell with other study samples. We cannot determine what the time frame will be to fill the Novaseq S4 flowcell.

Please fill a flowcell of interest to ensure your results are distributed as quickly as possible. If you only need one lane of sequencing, please contact us for the best options. If you have a deadline due to a grant, presentation, or paper, please contact the HTSF before submission.

Once a flowcell is filled and ready to run, an email is sent to the submitter approx. 24hrs before it will be loaded. See the attached table for the time period for a run to complete and data to be distributed. See Sequencing Timeframe Table in the *Forms and Guides tab, General.*

How will I know when my samples have completed sequencing?

You will receive an email from the HTSF customer service group when your samples are assigned to a flowcell, which typically means that the flowcell will be loaded within 24 hours.

Click here to view a Sequencing Timeframe Table which indicates how long it takes to sequence and download data. The information can be found in *Forms and Guide tab, Illumina.* Keep in mind that these are average timeframes and a run failure or barcode issue will delay data release. It there is a barcode issue is found at demultiplexing, HTSF customer service will reach out to submitter for corrections. The HTSF Bioinformatics group will also contact you with an email notifying you that your data has been distributed to your project folder and a link to the data.

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How will I know when my samples have completed sequencing?

You will receive an email from the HTSF customer service group when your samples are assigned to a flowcell, which typically means that the flowcell will be loaded within 24 hours. The Sequencing Platform Timetable indicates how long it takes to sequence and download data. The information can be found in *Forms and Guide tab, general.* Keep in mind that these are average timeframes and a run failure or barcode issue will delay data release. It there is a barcode issue is found at demultiplexing, HTSF customer service will reach out to submitter for corrections. The HTSF Bioinformatics group will also contact you with an email notifying you that your data has been distributed to your project folder and a link to the data.

QAQC

What instruments are used to perform the quality check (QAQC)?

The HTSF uses a variety of QAQC instruments such as the TapeStation, LabChip, and Bioanalyzer. Additionally, qPCR may be used depending on the library preparation method, such as 10x Genomics, of the sample. See *Submissions tab, Sample Quantification* for a detailed discussion about QAQC equipment.

My QAQC results show that my library sample contains two different fragment sizes or a broad range of sizes. What does this mean?

This means that it will be very difficult to accurately calculate the molarity of your sample. If the HTSF cannot accurately determine the correct molarity of your library, it can lead to under or overloading of the flow cell. You will be asked to decide which fragment size you want to choose for the calculation, or if you want to proceed. A sample exemption form may be required to be signed by the submitter's PI. With any customer prepared libraries, the HTSF cannot guarantee the quality of the sequencing results since we did not prepare the libraries.

My QAQC results show a failure or cannot proceed with my original library preparation request, what should I do?

The HTSF will contact you if your material fails QAQC or cannot proceed with the original library preparation request. The HTSF will also provide information on potential causes of the failure, advice on how to properly prepare your sample for resubmission, and alternative library preparation methods if applicable. The QAQC results will also be attached to your submission on TracSeq for your convenience.

Please contact the customer service team if you would like to schedule a meeting with the Research Director or Library Preparation Manager to further discuss your samples. You may also go the QAQC tab on the HTSF website for more information on QAQC interpretation, Common QAQC failures and the QAQC platforms available at the HTSF.

Library Preparation

Do I have to make my own libraries for submission?

While we will accept user-made libraries, we can also make the libraries for a fee. Please note that we do not guarantee successful sequencing of libraries not made by an HTSF technician. This is especially true for novel protocols. If you intend to perform a novel protocol, prior to submission, please contact Dr. Piotr Mieczkowski at piotr_mieczkowski@med.unc.edu. It is best to attach the protocol to your submission on TracSeq. If your protocol is custom, please select 'Custom' as your library preparation method in step 2 of the submission process. An empty cell will be provided for you to provide a brief description of your library preparation method.

What library preparation method should I use for my samples?

Your selected library preparation method will depend on your material type and project goals. Please contact the customer service team to schedule a meeting to determine the best way to proceed with your samples. Additionally, a rough estimate of library type, sequencing depth, and sequencing methodology can be found on the *Services* tab.

What are barcodes?

Individual "barcode" sequences are added to each DNA fragment during next-generation sequencing (NGS) library preparation so that each read can be identified and sorted before the final data analysis. A unique barcode is added to each library. These barcodes, or index adapters, can follow one of two major indexing strategies. The choice of Illumina adapters depends on your library prep kit and application. Please reach out to the HTSF if you need help determining what the best format is to use.

My libraries are dual barcoded. How should I show this information in the manifest?

When completing the manifest, type your barcode's nucleotide sequence under the IndexNT sequence column with no spaces or special characters. A hyphen will be used to separate the 2 barcode sequences. Please note that i7 is the first barcode followed by i5. There is no need to create a new column in the manifest. An example of a dual barcode you could type in the manifest is GTCAAGCT-ATCAACGT.

Note, do not reverse compliment (RC) any barcode sequences. If you submit as RC'd, the demux will fail and delay data delivery will be delayed. We realize that some platforms need an RC of the i5 barcode for the demultiplexing to work. Because platform for sequencing can change after submission and not all platforms require RC'd barcodes. Before demultiplexing begins, the HTSF database automatically checks what platform was run and if RC barcodes are required. The database will then RC if needed before running the demux. More information can be found in the Submitting Barcodes to HTSF guide on the *Forms and Guides tab, General.*

Is there a way to do a sequencing run quickly?

The HTSF does not offer "quick runs" for most platforms. We do, however, offer FAST_SEQ. This service is for the MiSeq ONLY. It requires you to speak to Tara Skelly or Amy Perou beforehand to have your account approved to make these requests. You will be preparing the sample fully to be loaded and supplying thawed, ready go kits for your run. These will be delivered on a pre-arranged day / time to the HTSF. We will load it on the machine, run the demultiplex and deliver data. You take all responsibility for the quality of the data. Only an Illumina confirmed machine failure will be run at no additional fee. You will only be billed for labor and limited consumables.

Please reach out to Tara Skelly and Amy Perou to discuss adding this to you available services. We review the necessary samples process that is required to happen prior to delivery to the HTSF. There is a FAST SEQ Release form which will need to be signed by the account PI and users/submitter.

Pooling

How should I pool my samples?

Your pooling plan will depend on your project goals, sequencing platform, organism, and required depth. Please contact the customer service team to schedule a meeting to determine

the best way to proceed with your samples. Additionally, a rough estimate-based library type, sequencing depth, and sequencing methodology can be found on the *Services* tab.

If you wish to learn how to best pool, please contact HTSF customer service. We will put you in touch with an HTSF production manager to assist you with best practices for pooling OR for rebalancing large numbered library pools prior to being run on a NovaSeq.

Sequencing

What is Next Generation Sequencing?

Next generation sequencing (NGS) is defined as technology allowing one to determine in a single experiment the sequence of a DNA molecule(s) with total size significantly larger than 1million base pairs (1millionbp or 1Mb).

Which sequencing platform should I use?

The platform that you should select depends on the number of reads you require. In the *Forms and Guides tab, Illumina* you will have access to a platform comparison chart. These tables will help you to determine which platform will best meet your data needs.

- Illumina Sequencing Platform Comparison Chart
- Illumina NovaSeq Standard Loading Sequencing Platform Comparison Table
- Illumina NovaSeq XP Loading Sequencing Platform Comparison Table

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Still unsure? Please contact the customer service team to schedule a meeting to further discuss your pooling and sequencing plan. Depending on your research goals, we may be able to create a pooling plan and select a sequencing platform that can save your project money.

What is the difference between the standard Novaseq and the NovaSeq XP?

The standard NovaSeq flowcell is designed to run a single pool across multiple lanes. This was the original way Illumina designed the NovaSeq platform to be loaded. The XP kit, however, allows for different pool to be run on each lane. To run the NovaSeq in XP mode required extra reagents, equipment, and time to load. Hence an additional fee needs to be applied to this run format. On the *Forms and Guides tab, Illumina* there is which will provide you with additional information:

- Illumina Sequencing Platform Comparison Chart
- NovaSeq Requirements and Pooling Suggestions

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What are the differences in data output between the HiSeq system and the NovaSeq and NextSeq platform?

The NovaSeq 6000 System offers output up to 6 Tb and 20 B reads in < 2 days. Multiple flow cell types and read length combinations offer flexible output and run time configurations based on project needs. In an evaluation of mRNA-seq, it was determined that systematic bias is expected when changing protocols from the HiSeq system to the NovaSeq. However, expected sources of bias – sequencing chemistry and patterned flow cells – did not broadly affect experimental results. Lastly, the magnitude of instrument bias is negligible relative observed biological variation.

On the *HTSF Landing page, What's New* there is a detailed review of the same libraries run on all platform and a comparison of data differences. The initial libraries were run on the HS2500v4 high output, then pools of those individual libraries were made and run on the progressively larger systems… HS4000, Nextseq. Novaseq. This study was initially run at UNC HTSF in early 2019 and then the Nextseq was run in 2021. The *Technologies tab, Illumina* for more information on the NovaSeq and other sequencing platforms.

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The initial study was between the HS2500 and the Novaseq. On the *HTSF Landing page, What's New archived posts* there is a detailed PowerPoint HiSeq vs NovaSeq comparison of data differences from the sample pools run HiSeq and NovaSeq.

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I have custom sequencing primers which are required for my samples. How do I submit my primers?

During step 4 of the submission process on TracSeq, you will be presented with the option to select that you need a custom sequencing primer. After selecting yes and 'Custom' for your primer, you will be able to provide primer name and sequence. You must complete this process for each submission. The HTSF does not store custom sequencing primers. Please follow the link here to the submission requirements for custom sequencing primers. The information can be accessed in the Submitting Sequencing Custom Primers to HTSF under the *Forms and Guide tab, General.*

If you require Old Nextera sequencing primer, please select this option during step 4 of the submission process on TracSeq. The HTSF keeps a stock of Old Nextera primer and therefore you are not required to provide this material.

What is Single-end vs. paired-end reading?

In single-end reading, the sequencer reads a fragment from only one end to the other, generating the sequence of base pairs. In paired-end reading it starts at one read, finishes this direction at the specified read length, and then starts another round of reading from the opposite end of the fragment. Paired-end reading improves the ability to identify the relative positions of various reads in the genome, making it much more effective than single-end reading in resolving structural rearrangements such as gene insertions, deletions, or inversions. It can also improve the assembly of repetitive regions. However, this degree of accuracy may not be required for all experiments and paired-end reads are more expensive and time-consuming to perform than single-end reads.

What is depth of coverage? How much do I need?

The depth of coverage is a measure of the number of times that a specific genomic site is sequenced during a sequencing run. In exome sequencing (WES), for example, the target might be 60X coverage, meaning that — on average — each targeted base is sequenced 60 times. This does not mean that every targeted base is sequenced every time; some segments may be read 100 or more times, while others might only be read once or twice, or not at all. In 60X exome sequencing our average target is that 85% of targeted bases are covered at least 15 times, and 90% of targeted bases are covered at least 10 times. The higher the number of times that a base is sequenced, the better the quality of the data.

For RNA-seq, we generally recommend a minimum of 30 million reads per sample. For sequencing projects that require higher accuracy — such as studies of alternate splicing — 40 million to 60 million paired-end reads will provide better results. For more detailed analyses to determine, for example, allele-specific expression or expression of low-abundant transcripts, 60 million to 100 million reads may be required.

What is cycle number (read length)?

During sequencing, it is possible to specify the number of base pairs that are read at a time. For example, one read might consist of 50 base pairs, 100 base pairs, or more. Longer reads can provide more reliable information about the relative locations of specific base pairs. This helps to address a common challenge that arises in sequencing because the same read sequences can appear in multiple places within a genome. However, it is usually more expensive to generate longer reads. If you require long reads, please contact the HTSF and discuss possible use of either BioNano or Oxford Nanopore. See the *Services tab, Long Read Sequencing* or the *Technologies tab, Long Read Technology*.

Why if PhiX added?

The HTSF adds the standard 1% PhiX that is required on all Illumina runs. If you suspect the complexity of the samples is low, additional PhiX may be required for the run to complete. See the Notes on PhiX Addition for Low Complexity Samples found on *Forms and Guides tab, Illumina.* The PhiX reads are moved prior to data delivery.

Consider your barcode selections prior to making your libraries if you are m using dual barcodes. Do not select the same i7 barcodes for all libraries to be pooled and only vary the i5. It is best to have i7 vary so the sequencing run will be successful.

What happens to my samples after sequencing is complete?

The HTSF will store submitted samples for 2 years. After 2 years, the HTSF will contact you to determine if you want your material to be returned or discarded. The HTSF will not store material after 2 years. Please contact the HTSF at any time for samples to be returned. The customer service staff is happy to arrange this for you.

Bioinformatics

I have been told my barcodes (indexes) are RC'ed (reverse complimented) and that my pool did not demultiplex (demux) properly. Why/how does this happen?

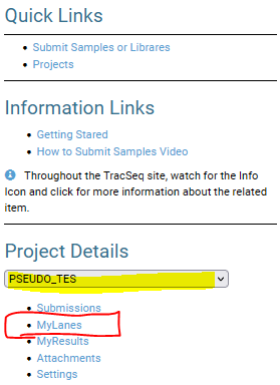
When i7 is RC'ed it is usually because the project used a PCR/Tagmented library preparation method such as Nextera. For i7's, clients should submit the barcode from the "sample sheet entry" column from their kit.

When i5 is RC'ed, it is usually because different instruments require the sequence as a forward read, while other instruments require it as a reverse compliment read. (see *My libraries are dual barcoded. How should I show this information in the manifest?* section above for further discussion). Because of this, we ask that clients give the actual sequence of their i5 in the manifest for their submission. Please note that when submitting barcodes on your TracSeq manifest, the i7 is the first barcode followed by i5.

The HTSF will fix your barcodes in our database and then rerun the demultiplex. This will delay the delivery of your data.

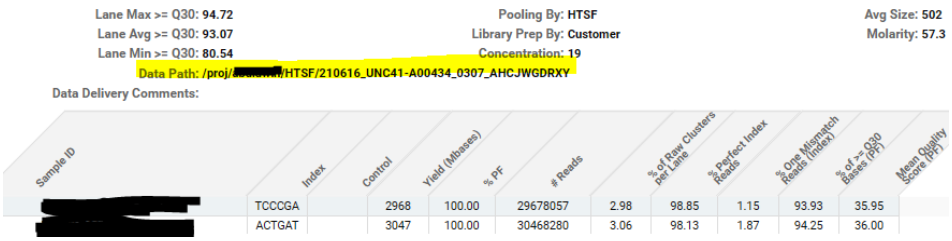
Where can I find my data as an UNC member?

For UNC studies, a data folder will be established on the ITS Research Computing server. If you do not have a folder, the HTSF can request one at the time of setting up your account at the HTSF. The HTSF will put the data in the HTSF subfolder for your lab. You will receive an email when we place data in the folder at to completion of sequencing. If you can ot find the email or feel you data should have been delivered already, use the MY LANES feature on tracseq.



- Open Tracseq and on the right side you will find the Quick links.
- Select your account and hit MYLANES below

- When MY LANES opens a new window, it will list all flowcells run for your account
- Use the name of your pool and the date to select the flowcell of interest *(note the FC are listed from oldest to newest. Use the search filters)*
- Hit the DETAILS button at the right.
- This takes you to a flowcell summary with run set up, run metrics, demux data amounts for each library in your pool, data delivery folder info, etc.
- Data delivery folder information is located just above the pool break out for metrics. I will list the folder and the run name *(highlighted below)*



Where can I find my data as an external account?

Once sequencing is completed, the data will be delivered to the google cloud. You will receive an email about the delivery and links to access it. The data MUST be downloaded to your organization's personal server within 5days. After which, it is deleted from the cloud. If it is required to be loaded again, there will be an additional fee for another 5 days. Be aware that some seq platforms yield terabytes (Tb) of data and it can take some time for the down load to be completed.

There is a small fee per Gb which will be billed to the project. UNC is billed to upload and store the data on the cloud. HTSF passes along this fee to the end users.

Can I get help analyzing my data?

Bioinformatics services can be completed in collaboration with the Bioinformatics and Analytics Research Collaborative (BARC), a new research support group in UNC-School of Medicine. BARC now includes personnel who provide critical bioinformatics support for UNC-HTSF. For more information on BARC, please contact Dr. Corbin Jones at cdjones@email.unc.edu and Dr. Hemant Kelkar at hkelkar@unc.edu.

Additionally, members of the Lineberger Comprehensive Caner Core, can contact David Corcoran, david.corcoran@unc.edu, for analysis services.

Grants, Papers and Presentations

I am writing a paper and need to confirm processing details. What do I do?

Please contact the HTSF customer service group with the information you need. It will help us if you can provide the account name, batch number of you have it, approximate date for the work and sample IDs if needed. We will confirm in the processing database what was done with your samples and can supply those details.

How do I acknowledge the HTSF on my paper, etc.?

> "We gratefully acknowledge the technical support from the UNC High Throughput Sequencing Facility. This facility is supported by the University Cancer Research Fund, Comprehensive Cancer Center Core Support grant (P30-CA016086), and UNC Center for Mental Health and Susceptibility grant (P30-ES010126)."

I am putting together a grant. Can I get a letter of support from the HTSF?

Most definitely. We would love to speak to you about your proposal. We may have some suggestions that can cut your cost and still meet your data goals. We do have a Requesting a Grant Letter of Support Form on the *Forms and Guides tab, Grant Writing*. Feel free to fill this

out to get the process started. All 48hrs, excluding the weekend, for us to get the form back to you. We can also supply quotes for work if you request.

I am working on a grant/paper and need HTSF Capabilities and/or a Rigor and Reproducibility statement for the HSTF.

Please go the About US tab on this site for a full discussion on these needs. There is a copy of the HTSF Capabilities *Forms and Guides tab, Grant General* and our HTSF Rigor and Reproducibility document on the *Forms and Guides tab, Grant Writing.*

Billing

How do I provide payment for HTSF services?

In step 1 of the submission process on TracSeq, you will indicate payment form: UNC chartfield string (CFS), purchase order, check (required for international studies). The Customer Service group will supply the study with an official quote (for a PO) or an invoice (international check) for use.

If you are a UNC researcher, you are required to use a chartfield string. Please contact your lab's accountant to determine your correct chartfield string. You may also confirm if your chartfield string is valid by going to https://chartfieldchecker.unc.edu/. An incorrect CFS will delay submission approval.

I put the incorrect chartfield string on my TracSeq submission. How can I change this?

If you have already submitted your samples on TracSeq, you will not be able to change your chartfield string. Please contact the customer service team and provide the correct chartfield string for your submission so the correction can be made.

How much will my submission cost?

Please contact the customer service team for an estimate of your requested services. A selected list of standard library preps and sequencing platform fees can be found on the *Fees tab*. If you know the services you require, you can send a Request A Quote Form to the HTSF. It is alos possible to submit via an on-line Request a Quote form available here. Please allow 48 hours for a returned quote. We are currently in the process of updating HTSF service fees. Fees for selected popular services will be posted once approved.