High Throughput Sequencing Policies

Experimental design:

Please contact us if you have any questions about designing your experiment. We provide consultation at no cost. Potentially, a simple consult could save thousands of dollars from a failed experiment.

Sample Queue

All samples are processed as they are received. We do not give priority to any sample based on investigator or institution. If there is a QC problem with one of your samples, we will contact you and wait for your decision to move forward, replace samples, or cancel. Once you have approved the QC, the sample/submission will be entered into the queue. Samples that are being repeated due to HTSF error will be placed at the top of the queue.

If you have an imminent paper, grant proposal or presentation, please let know prior to submitting so we can confirm we can meet your deadlines. These deadlines will be noted on submission batches.

Billing:

We generate invoices on a monthly basis. **We bill each month for work processed the previous month.**

- For **UNC investigators**, we require the chartfield string (CFS) number upon submission. If more than one CFS is required for payment, please discuss this with customer service BEFORE submission.
- For **non-UNC investigators** using PO payment, we require a hard copy of the PO with the submission to begin processing. We will generate an official quote for you to use to have the PO generated at your institution.
- For **non-UNC investigators** using Credit Card payment, we require the name and email for the person to contact for payment. We will send the invoice AND a credit card payment link to keep you information secure.
- For **international customer**, we require a check to be sent for payment for all services requested PRIOR to shipment of samples. We will generate an invoice for you to use to have the check generated. We will include the information for check payment to UNC at the time the invoice is sent. We request that you notify us when the check is sent and the check number.
- General processing billing note—when processing the submission request, a study may determine to discontinue (withdraw) further processing on some or all samples from a submission batch. The study will be responsible for payment for all work completed on samples at the time of samples withdrawal.

Please note that multiple invoices may be sent for a single project. For example, if library preparation and sequencing are requested for a single project, two invoices would be sent as these services will be completed at different times.

It is often requested that an invoice be sent immediately upon export of the data, so that money can be used from a grant that is expiring. In most cases, grants will continue to pay out after the date of expiration, as long as the service was completed within the grant timeline. HTSF lists the date of service on the invoices, so expedited invoicing is usually not required. Please notify HTSF customer service if you have a billing issue.

Publications:

If data generated by HTSF is used in an abstract, publication, or other formal communication, the submitter should include the following acknowledgement:

"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)."

The HTSF asks that you notify us of any acknowledgments you make on our behalf.

Sample retention:

HTSF will maintain excess samples for 2 years after completion of the study. If you would like to have that material returned, please contact us after you receive your data to set up a time to pick it up.

Completed libraries and pools (any remaining products from any of the services that we provide) will also be stored for 2 years after completion of the service. These materials can also be picked up upon request.

Annually, the HTSF reviews sample inventory. Accounts with samples older than 2 years will be contacted to confirm if they want material back or to be destroyed. A lack of response to this request means the samples will be destroyed.

Sequencing submissions:

We accept sequencing libraries generated in your own lab, or we can handle the entire process of library prep and sequencing for you. We can assist with QC of your starting material if you are planning to prepare your own sequencing libraries.

We require all samples be submitted with the correct submission information on Tracseq. Samples received without an approved Tracseq submission, will not be accepted or processed until complete information is provided.

We ask that samples be provided in 1.5ml tubes, with the label matching the sample name on the submission form. We accept Eppendorf style snap cap, screwcaps and matrix style tubes. See *Submission Tab* for container details. For direct to sequencing submissions, please include the Account Name and Batch # on the tube.

If submitting more than 30 samples at a time, please submit them in a 96 well plate. See *Submission Tab* for approved plate type. Please label the plates with the following:

- Account Name
- Batch number

- Date
- Plate Number

Quality Control for Next-Generation Sequencing (NGS):

- **Raw Materials :**

We realize that some labs do not have access to all possible QAQC equipment. A Nanodrop can be used if it is the only assay available. The HTSF does not prefer Nanodrop (or spectrophometer methods) due to the inaccuracies founds in the readings. Please see *Submissions Tab* for best QAQC practices and discussion on Nanodrop issues.

Upon receipt, all samples will be quality controlled based on submitted material type:
Genomic DNA– concentration assessed by Qubit, integrity assessed by Tapestation/LabChip
Total RNA– concentration assessed by Qubit, integrity assessed by Tapestation/LabChip
ChIP DNA– concentration assessed by Qubit, size range assessed by Tapestation/LabChip
Pooled Amplicons– concentration assessed by Qubit, size range assessed by Tapestation/LabChip

Please review the input values tables on the *Submission Tab*. We will contact you if your samples do not meet our input requirements. You may decide to:

- Withdraw the sample at this time and only be charged for the QC performed.
- To proceed with the prep, but you will be charged for the prep regardless of its success.
- To proceed with a different library prep method
- Withdraw and replace the sample. You will be charge for the initial sample QAQC

You will be asked to sign a sample exemption form (SEF) to confirm your decisions.

All libraries we prepare will be assessed for concentration and size. If we feel the library will not perform well when sequenced, we will send the library QC data to you to allow you to choose whether or not to go forward with pooling and sequencing.

- **Study Made Libraries**

Libraries that are submitted directly to be sequenced or pooled will have concentration assessed by Qubit and size range assessed by Tapestation/LabChip. We will use these values to calculate your molar concentration unless otherwise specified. If requested, QAQC for the libraries will be sent for your approval to move to sequencing.

If there is large presence of dimers, unusual size distribution, or if there is too little volume to achieve the required loading concentration, we will contact you to choose how to proceed.

- **Study Made Pools**

Pools which are submitted to the HTSF will have concentration assessed by Qubit and size range assessed by Tapestation/LabChip. We will use these values to calculate your molar concentration unless otherwise specified. If requested, QAQC for the pools will be sent for your approval to move to sequencing.

If there is large presence of dimers, unusual size distribution, or if there is too little volume to achieve the specified loading concentration, we will contact you to choose how to proceed.

Next-Generation Sequencing Specs:

For our standard runs, we offer sequencing per lane. Currently, we do not offer to split lanes between projects or investigators. Granted, you may organize that yourself among collaborators and submit the samples in a single submission. We will prepare an unbalanced pool should it be required. Contact customer service prior to submission if an unbalanced pool is needed. If you do not require a full HiSeq lanes worth of reads, we also offer MiSeq and Miseq Nano sequencing runs. Please review the *Technologies Tab* for platform details.

- **Run Issues and Guarantees:**

Adhering to Illumina-defined standards, if we obtain suboptimal read numbers or data quality due to an error on our part, we will rerun the lane at no additional charge to the consumer.

We do not guarantee sequencing quality or read numbers for libraries or pools that we did not prepare, non-standard library preps, or samples failing any of our initial QC metrics when the study has decided to move forward. This includes samples that are submitted to us for final QC quantitation and pooling, we cannot guarantee overall read number or individual sample reads.

The HTSF does have read ranges that we aim for on different platforms. Please see *Technologies Tab* for those details.

For sequencing runs that encounter an instrument error, we will contact Illumina's technical support. If they determine that the run or lane was a failure because of a machine or a reagent problem on the sequencer, the sample will be rerun at no cost to you.

- **PhiX Controls:**

We include a control PhiX spiked at 1% into every lane.

- For direct to sequencing submissions, if a library fails to generate clusters, but the PhiX library is observed, you will be charged for that lane.
- If no clusters are observed, we will contact Illumina's technical support to identify the run failure.
- If you request that PhiX not be used, you will be charged for the lane regardless of performance.
- For samples with poor base diversity, we recommend that you request a higher PhiX percentage to account for that, we cannot guarantee read numbers for imbalanced libraries.
- PhiX reads are removes prior to data delivery.

- **Custom Primers:**

We can accommodate samples using custom sequencing primers. However, these lanes are considered custom and will be charged to you regardless of performance. Details of the custom primer are required during submission to TracSeq. Please see detail on the *Submissions Tab*.

Custom primers are required to be submitted with samples by the study. A new tube of custom primer is required with each submission batch. We do not save any leftover custom primers. Please review Submitting Custom Sequencing Primer to the HTSF requirements document in the *Forms and Guides, White Papers.*

- The HTSF keeps a stock of OLD NEXTERA custom primer and it will not need to be supplied with your samples.
- If you require OLD NEXTERA, make sure to indicate in the custom primer section of TracSeq

- **Sequencing Guarantees:**

For unique libraries that are direct to sequencing samples, we ask that you suggest a desired loading concentration in your Batch Special needs notes on the submission. If you do not provide one, we will attempt to load the sample at a reasonable concentration based on the size range, type of sample provided for the requested run type and any historical work of the same type. We cannot guarantee optimal density particularly if the sample has an unusual size range. Subsequent submissions can be shifted up or down to better optimize density if you let us know the prior sample for comparison. We do not recommend mixing samples of different prep types onto the same lane as there is a size bias that could cause uneven representation of samples that is beyond our control.

Sequencing analysis:

The HSTF does not currently offer details sequencing analysis. We do review each run for quality before the data is delivered. There are different data delivery methods depending on your associations with UNC or if you are an external client. Please discuss the best option for your study with us if you have questions prior to submissions.

Bioinformatics forms an integral part of high throughput sequencing workflow. Should you require analysis of your sequencing data, there is a new group at UNC to assist you, BARC. Bioinformatics and Analytics Research Collaborative (BARC) is a research support group in UNC-School of Medicine. BARC now includes personnel who provide critical bioinformatics support for UNC-HTSF. We manage all data produced by UNC-HTSF starting with initial quality control through the final distribution. BARC personnel are available for advance consultation for researchers looking to get their samples sequenced at UNC-HTSF. BARC can also provide assistance with downstream data analysis after the sequencing is complete. Data analysis support is provided via a “fee for service” model for smaller projects. For large projects (both in terms of number of samples and duration) we can provide embedded and/or dedicated analysis and data management assistance.

For more information on BARC, please contact Dr. Corbin Jones at cdjones@email.unc.edu and Dr. Hemant Kelkar at hkelkar@unc.edu.

Data Deliverables

At this time the HTSF does not offer automated data analysis or pipelines. We are always here to help you with your data. Data is standardly delivered as FastQ. Please confirm if you need something else PRIOR to submission of your samples.

We do offer RNA data quality pipelines that can be requested to be run prior to data delivery. If you are interested in the use of these pipelines, Please reach out to BARC (https://www.med.unc.edu/barc/) and request fees to run the pipeline on your material. The cost varies with the extent of analysis requested.

Modern sequencers produce tremendous amounts of sequence in a single lane so samples are often mixed in a single lane in a process called multiplexing. During library construction, each sample is tagged with a unique index sequence (barcode) that is part of the sequencing adapter. Dual indexing (using two indexes) is also commonly used to tag samples. The informatics team "demultiplexes" the sequence data, assigning each read to the appropriate samples based on the barcodes. Demultiplexing (demux) is included in sequencing fees. Single and dual indexes are supported. Inline barcodes (index sequences that are attached to the library fragment and sequenced upstream of the inserts) are not supported. Inline barcodes will be sorted by traditional barcodes if they are included. Studies will have to do the final demultiplexing.

RNA-seq (model organisms and any other genome)

- FASTQ (default)
- BAM,
- counts table
- list of deferentially expressed genes (available for an additional analysis charge)

Whole Exome Sequencing (WES - model organisms and other genomes)

- FASTQ (default)
- BAM
- VCF (germline variants) (available for an additional analysis charge)

Whole Genome Sequencing (WGS - model organisms and other genomes)

- FASTQ (default)
- BAM (available for an additional analysis charge)

Data Retrieval

Once your sequencing is complete you will receive your data. Method of actual data release may vary based on your affiliation. Submitters are expected to download and keep backup copies of their own data.

- UNC affiliated --- data is delivery to UNC ITS Research managed data folders. If the end users does not know the directory for their folder, the HTSF will assist in setting up the folder. The PI is responsible for determining who will have access to the folder.
- Non UNC – data is delivered to Google cloud for retrieval with in 5days. This is the UNC required data delivery method. There is a fee for this service based on the Gb loaded to the Cloud. HTSF is charged by Google and we simply pass along those fees to the end user. If data is not down loaded by the user within 5days, HTSF will need to reload the data and additional fee will need to be charged.

UNC-HTSF maintains an archival copy of original sequence data for a period of up to three (3) years from the date data was originally released. If you need us to retrieve a copy of archived sequence data within

that period, a $300 data retrieval fee will be applied. Please note that there may be rare occasions when we are unable to retrieve data due to circumstances beyond our control.

HIPAA Compliance

Next-generation sequencing services at the UNC HTSF are for research only. All documents, emails, sample names, and data that you send through our submission form or by email must be HIPAA compliant. All sample names must be fully anonymized (no patient names).

Weather and Closure Policy

The HTSF is closed may be closed on occasion. We will always attempt to announce closures ahead of time. On occasion this may not be possible. The Website may not have last minute closure announcements, but Tracseq will contain them.

UNC Holidays:

- All UNC dictated holidays.
- Please refer to UNC calendar for established closures.

Weather Closures:

- The HTSF follows the UNC weather policy. In the event that the University opens late or closed, the HTSF will also open late or be closed for business.
- Weather events may affect our ability to maintain our usual data release deadlines.
- Read the special weather information to learn more about UNC's Inclement Weather Policies.

UNC Emergency Closures:

- The HTSF follows the UNC policy for all mandated closures.
- In the event that the University opens late or closed, the HTSF will also open late or be closed for business.
- Mandates may lower our production because limited staffing.
- These events may not have prior notifications.
- HTSF will post immediately on the TracSeq website information about closures. Website notification take longer to occur. But will be used for extended closure notices.
- Notifications will be updated as needed.

Staff Training:

- Announcements posted ahead of time on the HTSF website, the Tracseq submission site and on signs in the lab drop off area.