

TracSeq Step by Step Submission Guide

SUBMITTING SAMPLES AT THE HIGH THROUGHPUT SEQUENCING FACILITY:

The submission system for the HTSF is TracSeq and is a web based system that allows the user to submit samples for a project. It is ONYEN password protected. Your ONYEN is linked to specific project(s) that you have permission to submit to and view progress. If you do not see your project name on the drop down menu, please contact Erin or myself to have the project added. Do not try to create a new project. As you work through your submission, the system considers previous response to determine what to display for the next selection.

I have attached the TracSeq website for your next submission to the HTSF: <https://tracseq.genomics.unc.edu>. Below you will find a step by step guide of how to submit your material. If you still have questions, you may email Amy Perou <amy.perou@med.unc.edu> or Erin Wallace <wallacee@live.unc.edu>. Save any submission in progress. The HTSF staff will be able to see the submission in the design process and better answer your questions.

We look forward to working on your project
Amy, Erin, Kate

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Submitting on TracSeq

Step 1) Administrative Information:

After selecting your project from the drop down menu, the remaining cells will be prepopulated. All field remain editable.

1- Project:

- a. Select project from drop down menu
- b. The project is linked to the ONYENS for people who have permission to submit / view the project
 - i. If you do not see your project, contact HTSF. Do not try to create a project for submission.

2- Billing Account Type:

Previously submitted billing info will appear at the bottom of the selection field w/ the type of funding listed.

- a. UNC Account
 - i. Enter / edit the full chartstring via the blue link
 - ii. The asterisk fields are required and the standard start to any chartstring for a lab
 - iii. You will also need to list one for the following: Project Field OR Activity OR Program
 - iv. You may use the chartfield checker link (displayed at the top) to confirm the chartfield string. If there are issues, please contact your lab accountant prior to submitting for review.
- b. Purchase Order
 - i. An Official Quote can be sent several weeks prior for generating PO at home institution
 - ii. An electronic copy of the submitting university PO should be sent to HTSF before submission
- c. Check
 - i. For international projects and needs to be set up ahead of time
 - ii. A copy of the check transfer should be sent to the HTSF before submission
- d. Custom
 - i. This covers other funding sources, confirm with HTSF prior to submission
 - ii. enter the additional info in the box

3- Hit CONTINUE

- a. You will be asked if you wish to update the project defaults.

The screenshot shows the 'HTSF Material Submission' form in the tracSEQ system. The top navigation bar includes 'Dashboard', 'CRM', 'Sequencing', 'Bioinformatics', 'LIMS Pool Data', 'Work', 'Admin', 'FAQ', and 'Erin Wallace'. The form is titled 'HTSF Material Submission' and has three buttons: 'Save Work', 'Submit for Review', and 'Submit AS for Review'. The form is divided into sections, with the first section being '1. Administrative Information'. A note states 'All Fields are Required'. The form contains several input fields: 'Submitter First Name' (Erin), 'Submitter Last Name' (Wallace), 'Submitter Email' (wallacee@email.unc.edu), 'Submitter Phone' (919-966-3763), 'Assign Project' (Erin_Dev_Test), 'Principal Investigator Email' (wallacee@ad.unc.edu), 'Principal Investigator Name' (Erin Wallace), and 'Accounting Contact Email' (wallacee@ad.unc.edu). The 'Billing Account' section has four radio button options: 'UNC Account' (selected), 'Purchase order', 'Check', and 'Custom'. There is a blue link 'Add/Edit Chartfield' and a green 'Continue' button at the bottom right. A vertical 'Ask for Help' button is on the left side of the form.

Step 2) Source and Submitting Material

- 1- Select material you are submitting

- a. Samples (RNAs, DNAs, Amplicons, etc.)
- b. Study made libraries
- c. Study made pools

2- **Select library specifics** from the options

You will be presented with different options depending on what you select. Always make a selection in each field even if there is only 1 option. We attempt to have all possible library type listed whether HTSF made or Study made

- a. Starting source material
 - i. Please scroll through the entire list
 - ii. Notify the HTSF if the material you are submitting is not listed
- b. Library type
- c. Library prep method
 - i. Notify the HTSF if the material you are submitting is not listed

NOTE: If you find you, need to select CUSTOM at any point, please contact the HTSF before submitting for review.

3- Hit **Continue**

Step 3) Pooling (Multiplexing)

1- **Provided Material**

Click the option that best describes your samples needs.

- a. Samples pooled @ the HTSF
- b. Samples Pooled by the Study
- c. NO pooling needed

- i. For single library sequencing
- ii. For samples that will not be demultiplexed by the HTSF

2- Pooling Method:

NOTE: this will not appear if you are submitting study created pools

- a. Pooling by number:
 - i. Indicate the number you need in a pool in the box below
 - ii. HTSF will design the pools to meet the number per pool and barcodes regardless of the samples
- b. Pooling as set by project:
 - i. HTSF will pool based on information supplied on the manifest upload in step #7
 - ii. Project lists the same pool name so all samples that will be pooled together on the manifest

3- Barcode

- a. Not Specified
 - i. Select if you are submitting material for HTSF made libraries
 - 1. HTSF will determine the best barcode type when we make the libraries based on the prep method and the pooling requested.
 - ii. If you need to match previously made libraries, please contact HTSF before submission
- b. Single Barcode
 - i. Confirm from the libraries already produced
- c. Dual Barcode
 - i. Confirm from the libraries already produced

4- Hit Continue

The screenshot shows a web interface for submitting sequencing data. On the left, there is a vertical blue button labeled "Ask for Help". The main content area has a progress bar at the top with three steps: "1. Administrative Information", "2. Source and Submitting Material", and "3. Pooling (Multiplexing)". Below the progress bar, there are three columns of options:

- I will provide to HTSF:**
 - Samples that will be pooled by HTSF
 - No Pooling
- Pooling Method:**
 - HTSF will pool the samples in groups of
 - I will specify exactly which samples should be pooled together.

Pooling Details

To specify which samples will be pooled together, please include a "Pool Name" column in the per-sample details that you will provide in section 7 below.

The Pool Name maps each individual sample to a particular pool.

 - To Be Determined
- Barcode:**
 - Not Specified
 - Single Barcode
 - Dual Barcode

At the bottom left, there is a checkbox for "QC data approval before proceeding". At the bottom right, there is a green "Continue" button.

Step 4) Sequence Generation

1- Sequencing Set Up

- a. Make desired selections

Always contact the HTSF prior to submission if you are unsure of sequencing set up

- i. Sequencing Platform
- ii. Read Type Format
- iii. Read Length
 1. If you require custom cycle, additional field series will appear bases on previous selections
 2. Fill in cycle numbers for reads and index length (if used)

2- PhiX

Note that 1% PhiX will automatically be added to your samples.

- a. Select NOT SURE
 - i. If you are not sure if this is best for your experiment, select “not sure”
 - ii. HTSF will determine the amount required based on the library type and QAQC of your samples.
- b. Select YES
 - i. If you know from previous work it is required. Typically, PhiX % is increased with low complexity samples
 - ii. In Step # 7, Batch Special Needs, you need to list “PhiX= *your known PhiX %*”
 - iii. You will also need to list this on the manifest
- c. Select NO
 - i. You know you do not need more than standard 1% PhiX

3- Sequencing Primers

- a. If you are submitting Libraries or Pools, you will be asked about the need for custom primers. Select on the drop down:
 - i. Old Nextera Primer
 1. Nothing more is needed, HTSF maintains this Old Nextera Primer in house
 - ii. Custom - you will need to supply:
 1. Custom primer(s) name
 2. Custom primer (s)sequence
 3. Study must supply custom primer when dropping off samples.
 - a. Primer tube **MUST** be dropped with each submission
 - b. Label the primer tube with
 - i. Study Name
 - ii. Batch Number
 - iii. Submitter Name
 - iv. Primer Name

4- Hit Continue

4. Sequence Generation

Select Platform:

HiSeq 2500 High Output	HiSeq 2500 Rapid Run	HiSeq 4000 High Output	MISeq	MISeq-Nano
NovaSeq 6000 S1	NovaSeq 6000 S2	NovaSeq 6000 S4	NovaSeq 6000 S1 XP	NovaSeq 6000 S2 XP
NovaSeq 6000 S4 XP	<input checked="" type="checkbox"/> NovaSeq 6000 SP	NovaSeq 6000 SP XP	No Sequencing	

Read Type Format:

Paired End

Read Length

2x50 2x100 2x150 Custom

Illumina SOP indicates 1% PhiX should always be added to a run. This is regardless of platform.

Is more than 1% PhiX required?

Yes (more than 1% PhiX is required)

No (1% PhiX is sufficient)

Not Sure

Continue

Step 5) Container and Diluent

1- Container Type

- Select the type of container you are submitting your samples in
- Accepted container types are displayed. There are short descriptions of container requirements listed below.
 - Plate submission:
 - HTSF ONLY accepts plate type listed. Contact HTSF if you are unable to supply this plate
 - If you are submitting more than 24 samples, we recommend using a plate instead of individual tubes
 - Make sure to list sample order on the submission manifest in column format. Robots do not work in row format. If you have questions contact HTSF
 - Matrix tube selection, you will be asked for the 2D barcode to be listed on the manifest you upload in step #7

2- Diluent

- Acceptable diluents are listed with short descriptions below
- Absolutely NO TE is allowed. TE/ EDTA prevents sequencing of the samples.

3- Hit Continue

4. Sequence Generation

5. Container and Diluent

Container:

<input checked="" type="checkbox"/> Snapcap1.7	<input checked="" type="checkbox"/> Elution Buffer
96 Well Shallow Plate	dH2O
MATRIX0.5	No Dilution
Slide	

Diluent:

Elution Buffer

dH2O

No Dilution

Diluent Requirements(Sample Solution) : Elution Buffer (EB) is defined as Tris-Cl, pH ~8 (no EDTA). Note that HTSF cannot accept TE as a diluent since it contains EDTA and degrades the source material.

Required Containers: Tubes must be 1.7 mL snapcap or screwcap (Eppendorf-style). Plates must be BioRad 96 well Skirted PCR plate, hard-shelled (Catalog number: HSP-9631). We cannot accept: 0.5 mL or 2mL Matrix tubes, Dolphin tubes, or any tubes ≥ 2.5 mL.

Continue

Step 6) Informatics

- 1- **Data Delivery Notification** – emails will be sent to these individuals
 - a. Default data delivery person will be listed
 - b. Additional lab members can be selected
- 2- **Data Delivery** –
 - a. There are a variety of options to choose
 - b. When ITS Research Computing Storage is selected, the folder the data
- 3- **Alignment** – if you would like the alignments done on your data, please select form the drop down menu. We list the common alignments we can do. We do not have all possible alignments.
- 4- Select if you want a **consultation** from the Center of Bioinformatics
- 5- **Comments:** any information you need passed on the IT staff should be placed here.
- 6- Hit **Continue**

6. Informatics

Data Delivery Notifications:
No default members to assign.

More Members 1

Erin Wallace - wallacee@email.unc.edu

How would you like to retrieve your data from HTSF?

- Deliver my data to ITS Research Computing storage (/proj)
- Deliver my data via LCCC Data Delivery Pipeline
- Provide me with a URL to download my data via the web
- Pre-arranged data delivery method for this project
- Other

Alignment (Reference Genome)

Mus Musculus 9

Would you like HTSF to perform downstream data analysis?

- Yes (Contact Hemant Kelkar -kelkar@unc.edu- to discuss before you submit samples)
- No (Only raw sequencing data will be returned.)

Comments:

500 of 500 characters left

Continue

Step 7) My Sample Details

Sample Manifest Submission:

You will submit a manifest that describes each of your samples. The information that HTSF needs for each sample is different depending upon the selections you have made in the previous steps. Please complete all fields

NOTE: you will not be able to access this section until you have completed ALL of the sections above. A check mark will appear for all completed selections.

NOTE: if you do not have all QAQC values:

- List 9999 in the field. This lets us know it is unknown.
- Volumes and conc are required to be listed
- HTSF always reruns QAQC. We will contact you if there are issues with the samples and discuss options to proceed.
- Place note in Batch Special Needs (step #7) if you want the QAQC to be sent prior to processing

1- Down Load Manifest

- Click the **“Download manifest template based on selections”** link, which will provide you with an excel spreadsheet based on your previous selections.

2- Complete the manifest, save

- If you have notes for individual samples, please list them here for the staff.
 - If your note applies to all samples, List it in Batch Special Needs at the end of this step
- If you are submitting a plate, list the samples in Column Format: A1, B1, C1 --- H11, H12
- If you are submitting Matrix tubes, list the 2D barcode for each samples. We will be using this for confirmation in our facility.

3- Browse and upload manifest

Ask for Help

4- You will see the **manifest displayed on the TracSeq** page

- If there is an error, a message will be displayed
 - Make modifications to the manifest outside of TracSeq
 - Most common error:
 - Sample ID is not 1-16characters long
 - Sample ID or external code contains special characters or spaces. You may use underscore or hyphen to separate sections of IDs
 - Missing QAQC info
- You can **CLEAR and RESET** the current manifest and reload the modified manifest
Contact HTSF if you cannot figure out an error issues

- 5- Add any comments to the **Batch Special Needs** field that applies to all samples submitted. These notes will be displayed to the techs as they work.
- 6- Hit **submit for HTSF review**

*Email is sent to the HTSF for review

*Allow time for the review process to be completed before drop off or shipment. Time typically varies from a few hours to 24hr to complete

Submitting for Review

After clicking “Submit for Review”:

Your submission will be sent to the HTSF.

- 1- Once the batch submission has been reviewed, you will receive an **ACCEPTED NOTIFICATION EMAIL**. If there are any issues, we will contact you.
- 2- HTSF will generate a quote for the service fees to complete the work requested. And email is sent to you with his quote
- 3- In the **QUOTE NOTIFICATION EMAIL**, you will find a link to finalize the submission
 - a. Open the link
 - b. Indicate the estimated date you plan to drop the samples.
 - c. Print out the confirmed manifest
 - i. If you need to reprint the manifest at a later date:
 1. Open TracSeq
 2. Open Projects Link
 3. Select batch you need manifest for
 4. Select View Drop Off Manifest
 5. Print
- 4- **Sample delivery:** Bring the confirmed manifest with your samples to the HTSF
 - a. Genome Sciences Building, rm 1153
 - b. Sample drop off Monday – Friday / 9-4:30
 - c. Phone # 919-962-4395
 - d. HTSF is closed for all UNC holidays