

Rigor and Reproducibility at the HTSF

Overview of UNC Mandated Core Rigor and Reproducibility

Purpose: NIH research grant and mentored career development award applications must address rigor and transparency requirements outlined in the application instructions. Research Performance Progress Reports (RPPR) must emphasize rigorous approaches to ensure robust and unbiased results. The information below should help provide the necessary information to investigators on the best practices to assure rigor and transparency in research performed at the HTSF. The first section provides general guidelines and the second section provides HTSF-specific guidelines.

Rigor means following procedures that will increase the likelihood of obtaining an accurate representation of the phenomenon under study.

Reproducibility means recording and communicating those procedures such that they can be replicated accurately.

You may use this as a template for wording in your grants and publications to address Rigor and Reproducibility for your sequencing experiment run at the High Throughput Sequencing Facility.

Eight steps to Rigorous and Reproducible Experiments in Biomolecular Research at UNC:

1. If using a core facility, **consult** with the core staff in the planning stage. Consult with a statistician if you need help developing a Power Analysis to assure that your results will be adequately powered.
2. Design your experiment with **sufficient controls** (rigor) **and replicates** (reproducibility).
3. Assure that ALL of your reagents (antibodies, cell lines, mice) are **fully validated** (see below).
4. Have a clear and **detailed protocol** (SOP) and data analysis plan. Assure that the protocol is strictly followed or that any deviation is well documented.
5. Assure that the staff or students performing the experiment are **well trained** and understand each step and the importance of performing them precisely.
6. Use only **well-maintained instrumentation**, preferably maintained and operated in a core facility with expert staff (see #1 above).
7. **Document all steps**, reagents, equipment and data analysis methods used in the experiment. Assure that the both the documentation and the data itself are properly stored in a safe data management repository.

8. **Acknowledge** the Cancer Center Support Grant (*P30 CA016086*) (*if applicable*), other grants that support the core, the core (by name), and core staff in publications.

HTSF Rigor and Reproducibility Guide

Project Planning

Please contact the following for assistance with establishing a study at the HTSF:

Piotr Mieczkowski, [Piotr Mieczkowski@med.unc.edu](mailto:Piotr_Mieczkowski@med.unc.edu)

- Technologies inquires
- Study planning for all technologies
- Novel applications
- Oxford Nanopore
- BioNano

Amy Perou, amy_perou@med.unc.edu

- Illumina based study planning
- Project set up / updates

Tara Skelly, tskelly@email.unc.edu

- Oxford Nanopore

Ewa Malc, ewa_malc@med.unc.edu

- BioNano, Saphyr

Controls and Standards

Noteworthy HTSF practices that address guidelines include the following:

- Maintenance of a QMS of Facility Standard Operating Procedure (SOP)
- Maintenance of a QAQC SOP
- Requirement for a signed study initiation document between the HTSF and the project PI/Staff before work can begin
- Study PI signed SAMPLE EXEMPTION FORM (SEF) is required for samples that deviates from
 - the original submission request
 - QAQC values required by HTSF for a procedure
 - Electronic version of SEF is attached to the submission batch records for the study to view. Hard copy versions are retained.

- Maintenance of individual equipment maintenance standard operating procedures (SOP)
- Maintenance of individual specimen processing protocol SOP
- Use of a submission database, TracSeq, to record:
 - Study request for sample processing
 - Notations of study updates
 - SEF and QAQC data attachment for study reference
 - Sequencing tracking with run metrics and data delivery information
- Use of a Laboratory Information Management System that maintains
 - An audit for each sample processed in the system.
 - Provides data QAQC control by validating data entered into the system.
 - system's database is backed up on SOM servers
- Freezer maintenance and back up policies.
 - All freezers are alarmed with automated call systems to HTSF staff

Reagent Validation

- Commercial standards are run on all DNA/RNA quantitation instruments on every run. When originally purchased these standards are validated on more than one instrument
- Positive and negative controls are incorporated into runs as appropriate but at a minimum when a new lot of equipment commercial-purchased reagents are used.

HTSF Protocols

HTSF keep a detailed list of Standard Operating Procedures (SOP) which includes all processing protocols. These are set up in a QMS manner and are updated as deemed necessary by group managers. Available for inspection upon request

HTSF Staff Training

HTSF staff members complete all UNC EHS training modules, CITI training modules when initially hired and on an annual basis as required

In addition, HTSF staff are required to read and become certified by their appropriate group managers for any SOPs that is developed/updated OR new to the staff member prior to use. A record is kept of all QMS SOP certifications for all SOPs.

There are no professional certifications required at the HTSF

Instrumentation Maintenance

Every instrument used in the HTSF has a maintenance SOP that describes weekly, monthly, or longer maintenance tasks. Running hard-copy (attached to each piece of equipment) and electronic logs are preserved. Electronic logs are saved on a secure server that is backed up and maintained by the SOM. In addition, major pieces of equipment are covered by maintenance agreements with either the original vendor or UNC approved third-party vendors. Yearly preventative maintenance tasks are performed by certified technicians.

Documentation

HTSF has extensive documentation several areas. Each area has an electronic record. All systems and electronic records are maintained UNC maintained service which are backed up every 24hrs.

- QMS SOPs
 - All processing protocols are in QMS SOP format
 - All SOPs are reviewed biannually for necessary updates
 - SOPs are constantly updated on an as needed basis
 - QMS SOPs also cover all lab operations beyond protocols:
 - Equipment
 - Facility and Safety
 - Bioinformatics
 - Assessments of the Core
 - Customer Focus
 - Documentation
 - Continual Development
 - Information Management and Data Security
 - Non-conforming Events
 - Organization
 - Personnel
 - Process management
 - Purchasing and Reagent Inventory.
- Training
 - See above
- TracSeq database
 - System is ONYEN authenticate to gain access (UNC password protected)
 - Maintains User and account records
 - Records the submission requests of all materials
 - Records all communications with HTSF users
 - Allows for document attachment to an account for users access
 - Audit records with dates/ protocol / staff member performing task are available for all work performed on ever sample at the HTSF
 - Maintains all Sequencing records
 - Design of flowcells with assignments of samples
 - Run names
 - Details of the run, including run issues
 - Metadata for the run
 - Demultiplexing of the data for each sample run
 - Data delivery
 - Records are available for HTSF users to view
- LIMS database
 - HTSF production database, HTSF users do not have access to LIMS
 - System is ONYEN authenticate to gain access (UNC password protected)
 - Maintains sample list for accounts
 - Assigns unique identifier to each container received and produced at the HTSF
 - Records all procedures performed on materials received at the HTSF

- Audit records with dates/ protocol / staff member performing task are available for all work performed on every sample at the HTSF
- Maintains all samples records as to
 - Use, volume, QAQC
 - Downstream products of each sample
 - Storage

Acknowledgement and Grant Support

Please indicate the following acknowledgement for all papers, grants, posters and talks:

“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).”