UNC HTSF Capabilities

Overview

UNC's High Throughput Sequencing Facility offers a multitude of services including a broad range of Illumina sequencing technology, long read technology, library preparation and data interpretation. Encompassing multiple floors within the Genome Sciences Building on UNC's campus, the HTSF is sure to meet your research needs.

Facilities

With over 4900 sq. ft. of lab and office space, the HTSF is equipped to handle large scale research projects. Operating over several floors of the Genome Sciences building, the HTSF houses many different technologies to allow for a broad range of services. The HTSF is comprised of knowledgeable staff members including library preparation, sequencing, bioinformatics, technology, customer service teams that are happy to help advise projects on how best to process their samples. We also have a research and development group that continually work to bring new technologies to the HTSF and work with studies on novel use for current technologies. HTSF continues to be a beta test site for a number of local and international partners.

Illumina Sequencing

The HTSF has several different Illumina sequencing technologies. With the many sequencing options available, the HTSF can offer novel ways to achieve the desired amount of data. Please refer to our Technologies tab on the HTSF website for more detials.

Please refer to our Service tab and/or Technologies tab on the HTSF website for information on sequencing at the HTSF.

MiSeq

The MiSeq Platform is capable of covering up to 20M reads per lane. It is very versatile with a number of different cycle versions that can standardly be run and can easily be manipulated to run unique or custom cycle formats. The HTSF has observed that the Miseq is more forgiving in terms of library quality when compared to other systems, such as the HiSeq 4000. The ability to run custom cycles and library quality flexibility makes the MiSeq especially helpful with R&D library development or pilot assays. It is

the workhorse for a variety of applications, including targeted gene, small genome, and amplicon sequencing, ITS and 16S metagenomics. Additionally, we offer Miseq Nano runs which serve well to run QAQC on new protocols, checking balance on a pool or when only minimal data is needed (1Mreads/ run).

HiSeq2500

The HiSeq 2500 System is a powerful high-throughput sequencing system. Currently, other than legacy studies, the HiSeq2500 allows for 2 lanes to be run in the rapid mode. It can also be run in the v4 high output mode, allowing for 8 lanes of sequencing. This is typically only used for legacy studies.

Please note: The HiSeq2500 System is now considered obsolete by Illumina. They intend to continue to provide full support of the instrument and supply the reagents through February 28th, 2023. Because of the HiSeq2500 status with Illumina, reagents have become more expensive. Please inquire about other options.

HiSeq4000

The HiSeq4000 System uses a patterned flow cell technology to provide rapid, high-performance sequencing. It has greater data capacity per lane over the HiSeq2500 system and remains at 8 lanes per flowcell. It is the go-to technology for many studies that do not need the capacity of a NovaSeq SP flowcell or studies which are completing a legacy project.

Please note: Illumina will be discontinuing the HiSeq4000 Systems. They will continue to provide full support of the instruments and supply the reagents through March 31, 2024. Because of the HiSeq4000 status with Illumina, reagents have become more expensive. Please inquire about other options, especially prior to beginning a long-term study.

NovaSeq6000

The NovaSeq6000 is currently the most powerful Illumina sequencing platform. There are multiple flowcell versions which each have different data yields per lane. The NovaSeq works well for large production studies, deep sequencing, whole genome sequencing, whole exome sequencing, RNA sequencing and tumor-normal profiling.

There are several flowcell options for you to choose from. Depending on the depth of data needed, certain flowcells may work better for you. The HTSF is here to help you determine the best format for your study's data needs. Please refer to our Technologies tab on the HTSF website for more information on flowcell choices.

Alternative Technologies

In addition to our Illumina technologies, the HTSF offers several options for the processing and sequencing of unique samples. With these alternative services available, the HTSF can meet a broad range of needs and study goals.

Alternative Library Technologies

The HTSF is excited to now offer a growing range of genomics applications for single cells, including RNA-seq, gene expression profiling by qPCR and DNA amplification for whole-genome or targeted (exome or PCR-based analysis) through the **10x Genomics Chromium** platform.

This technology supports a broad range of applications, including cancer-cell transcriptomics and cell-type identification and discovery. Because the platform works with short read sequencers, it integrates easily into the existing HTSF sequencing workflow allowing for an end to end service. Please refer to our Technologies tab on the HTSF website for more information on our 10x services.

The HTSF is also offering the **HTG Molecular's EdgeSeq.** This technology is a fully automated sample and library preparation platform for targeted RNA sequencing that pairs HTG's extraction-free, high-specificity Edge Chemistry with the high sensitivity and dynamic range of next-gen sequencing. EdgeSeq enables digital quantitation of miRNA and mRNA expression from difficult sample types such as formalin-fixed, paraffin-embedded (FFPE) tissues, plasma and exosomes. Please refer to our Technologies tab on the HTSF website for more information on our HTG assay services.

Please note that both 10x Chromium and HTG EDGE libraries are run on traditional Illumina sequencing platforms.

Long Read Technologies

Oxford Nanopore Technologies offers direct sequencing of native DNA or RNA, or samples that have been amplified with PCR. Nanopore sequencing can read any length of DNA/RNA, from short to ultra-long. It is useful for applications such as *De novo* assembly, scaffolding and finishing, bridging repetitive regions, structural variation, SNVs and phasing, targeted sequencing, RNA analysis, metagenomics, and epigenetics. The Oxford Nanopore's unique direct molecule sequencing platform is based on protein nanopores set in a polymer membrane. As DNA or RNA pass through the current-filled nanopore, the current is disrupted, and the DNA or RNA are detected. Please refer to our Technologies tab on the HTSF website for more detials.

The HTSF also offers optical mapping with the introduction of the **Bionano Saphyr** instrument. Optical mapping can detect large-scale structural variations ranging from

500 bp to megabase pair lengths. Structural variations are responsible for many diseases, including cancer and developmental disorders. One Saphyr flow cell can produce up to 1300 Gb of data. Please refer to our Technologies tab on the HTSF website for more detials.

Library Preparation

The HTSF has access to several different library preparation methods to accommodate a wide variety of samples types based on the needs of the project. Besides offering many options for library preparation kits, the HTSF is also able to perform custom library preparation. Custom library preparation requires a consult before the submission of samples to confirm the best methodology to accomplish the project's goals. Please refer to the HTSF Capabilities: Library Preparation Services document for a thorough list of all these methods.

The most common library preparation methods include:

- DNA Kapa Hyper
- Kapa Ribo Erase for Total RNA
- Kapa mRNA
- 16s Metagenomics
- TruSeq Ribo-Zero Gold for Total RNA
- Small RNA preps such as BioO Scientific and HTG Edge
- SMARTr XT plus Nextera
- Tecan Genomics/Nugen suite of libraries (great for non-standard, model organisms)

Quality Control and Quality Assurance

The HTSF strives to provide the best possible data to ensure each project's needs are met. To do so, the HTSF performs several QA/QC options when processing samples in preparation for sequencing. The HTSF will always perform QA/QC on materials arriving at our facility unless specified otherwise. This is regardless of previous QAQC performed by a study or another core.

qPCR

The HTSF uses quantitative PCR works to calculate a concentration by amplifying and fluorescently tagging each DNA strand and measuring the change in tagged molecules after each cycle.

Tapestation

The Tapestation is an automated electrophoresis tool the HTSF uses for both DNA and RNA quality control. The machine analyzes size, concentration and integrity of samples submitted to our facility. This platform is equivalent to the BioAnalyzer, but has great flexibility in number of samples loaded in a single run.

LabChip

The LabChip is another automated quality control machine that quantifies and measures the size of both DNA and RNA. The HTSF uses this service when many samples are being measured at once.

Qubit

Our Qubit Fluorometers are designed to precisely measure the concentration and presence of DNA in an entire sample. This service is used to determine and accurate concentration for QA/QC. HTSF finds this to be significantly more accurate than a Nanodrop.

Fragmentation Services

The HTSF is proud to offer the use of our LE220R-plus Covaris Sonicator to laboratories outside of the HTSF. The sonicator is available for use to any laboratories on UNC's campus. Simply schedule a time with the HTSF during operational hours and have full access to the machine during the designated time slot. You will be expected to supply all consumables for use. There is no fee for this service.

BSL2 Abilities

The HTSF has BSL2 room for our exclusive use. Our staff is trained to work for sample processing that requires the extra level of security. We can work with mammalian cells, bloods and other body fluids and deactivated viruses. The room is equipment with a full suite of instrumentation to carry an experiment it's end point: Laminar flow hood, HTG, QAQC services, 10xGenomics, etc.

This BSL2 room allows us to perform experiments on live mammalian cells for single-cell experiments as well as extract DNA and RNA from solid tissue and body fluids. Additionally, we are able to prepare sequencing libraries from non-infectious DNA or RNA material derived from pathogens including SARS-Cov-2. These viruses need to be heat or chemically inactivated in a BSL3 or higher prior to our receipt.