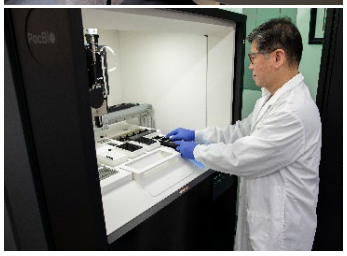
Network Biology Collaborative Centre

Sinai Health | Lunenfeld-Tanenbaum Research Institute

NBCC Next-Generation Sequencing (NGS) Guide

The Network Biology Collaborative Centre (nbcc.lunenfeld.ca) at the LTRI provides both short and long read sequencing capabilities for the analysis of gene function and expression. This guide provides information on the sequencing applications we offer and the instrumentation that is available for analysis.

I. Who we are



The NBCC NGS node is overseen by LTRI's Senior Scientist and NBCC co-Director Dr. Jeff Wrana who is an expert in single-cell and high-throughput NGS sequencing applications. The facility is managed by Kin Chan who has over 15 years' experience in sequencing technologies with a focus on transcriptomics profiling. He is assisted by sequencing specialist Angeline Zhang who is an expert in sample preparation and operation of NGS instruments. The NBCC Director of Operations Dr. Karen Colwill provides administrative and logistical support.

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Research Resource Identifier (RRID): SCR_025385

II. How we help

The NBCC provides full support for your sequencing needs from initial study concept to manuscript preparation and data submission to public repositories. Our service starts with an initial consultation to identify the best and most cost-effective approach to your project. Our services include sample preparation, quality control, sequencing, bioinformatics support and assistance with grant and publication writing related to the services we offer.

III. Core instrumentation

The NBCC provides both short-read (up to 600 bp in length) sequencing using Illumina second generation instruments, notably the NextSeq 2000, and long-read sequencing (up to 15-20 kb in length) using a PacBio Revio instrument (the first in Canada). The NextSeq 2000 is a versatile mid-range capacity sequencer ideally suited for rapid turnaround of small to medium-scale assays for gene function analysis (e.g., CRISPR screens), technology development, and more customized applications.

In addition to the sequencers, we have a 10X Chromium for isolation of single cells or nuclei prior to sequencing and a GeoMx digital spatial profiler for transcript analysis direct from tissue. These instruments along with other instruments dedicated to sample preparation and quality control are at the heart of the services that we provide.

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IV. Applications

A. Gene function analysis

CRISPR-based functional screens (gene knockout or activation) connect changes in genotype to phenotypic output and are commonly applied to find genes essential to a particular experimental condition. In these assays, a pooled lentiviral single guide (sg)RNA library is used where each sgRNA is identified by a unique barcode. Typical screens involve endpoint reads where changes in sgRNA frequency in the cell population across different experimental conditions are identified by NGS. Analysis is performed using an Illumina short read sequencer to identify and quantify the barcodes for each unique sgRNA. Confirmation of on- and off-target integrations, if required, can be performed using long-read sequencing on the PacBio Revio system. This will also identify any genetic variations, such as single nucleotide variants (SNVs), that have introduced allele-specific Cas9 cleavages.

B. Whole genome sequencing

We provide whole genome sequencing capabilities using either short or long read sequencing. Short read sequencing provides higher depth, is typically cheaper, and is still an effective route for analyzing well-characterized genomes. Long-read sequencing using the PacBio Revio offers the advantages of identifying complex structural variations (large insertions/deletions, inversions, repeats and duplications, and translocations) and is best suited for de novo genome assembly and for phasing single nucleotide polymorphisms (SNPS) into haplotypes.

C. Bulk gene expression profiling

Bulk RNA sequencing (RNA-seq) from cells, tissues or organoids enables gene expression profiling between different conditions. Whole transcriptome, whole exome, or targeted sequencing options are available. For differential expression analysis, transcripts (after conversion to cDNA) are sequenced using short-read technology to identify and quantify RNA expression levels. For mapping of full-length isoforms to identify splicing variants or alternative start and end points of a transcript or to assign SNPs to specific isoforms, the analysis is performed using our long read PacBio Revio sequencer.

D. Single cell biology

Isolation of single cells or single nuclei prior to RNA or DNA sequencing enables gene expression, chromatin structure, and copy number alterations to be assessed at the single cell level within mixed cell populations. Lineage tracing can also be performed using barcoding. The 10X Chromium platform available in the NBCC is the core technology that enables easy and efficient partitioning of individual cells for single cell mapping.

E. Spatial profiling

We offer spatial transcriptomic profiling of both formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissue using Nanostring's GeoMX digital spatial profiler coupled to Illumina sequencing. Regions to profile are selected based on fluorescent staining patterns of selected markers. With the newly awarded CFI to Wrana and Pelletier, we will be acquiring 10X Xenium and Visium HD capabilities from 10X Genomics.

F. Multiplexed functional screens

Dr. Jeff Wrana in collaboration with Dr. Ben Blencowe (UofT) developed a multiplexed and quantitative functional genomics screening platform that enables screening in multi-well plates (e.g. different drug conditions or siRNA screen), barcoding of samples and NGS readout of changes in gene regulation (it was first used to monitor changes in alternative splicing (Han et al. Molecular Cell, 2017)). A variation of this assay, C19-SPAR-Seq, was developed to track SARS-CoV-2 variant dynamics.

G. Pathogen detection and profiling

NGS is ideally suited to detect novel and existing pathogens in an unbiased manner through metagenomic or metatranscriptomic sequencing with the benefits of being able to identify potential co-infections and follow host response to infection. In addition to unbiased screening, different strains of bacteria can be identified through 16S/internal transcribed spacer (ITS) ribosomal RNA sequencing and other targeted methods (including variations of SPAR-Seq as noted above).

H. The “latest acronym”-Seq

New applications and approaches to sample preparation and sequencing analysis are continually being developed most of which are identified by an acronym (e.g., ATAC-Seq to identify transposable-accessible chromatin, ChIP-Seq for chromatin immunoprecipitation followed by sequencing to profile DNA-binding proteins….). If you have an application of interest, have heard of a specific technology, or have an idea you want to discuss, please contact us.