Network Biology Collaborative Centre

Sinai Health | Lunenfeld-Tanenbaum Research Institute

NBCC High-Throughput Screening (HTS) Guide

The Network Biology Collaborative Centre (nbcc.lunenfeld.ca) at the LTRI provides automated solutions for the high-throughput (HTP) analysis of gene function and early drug discovery. This guide provides information on the screening applications we offer and the instrumentation that is available for analysis.

I. Who we are



The NBCC HTS node is overseen by LTRI's Senior Scientist and NBCC co-Director Dr. Jeff Wrana who is an expert in developing and executing screening strategies. The facility is managed by Jenny Wang who has over 25 years' experience in laboratory automation and executing HTP screens. She is assisted by automation specialists Mariam Iskilova and Mark Jen who are experts in developing and implementing robot-assisted technologies. The NBCC Director of Operations Dr. Karen Colwill provides administrative and logistical support.

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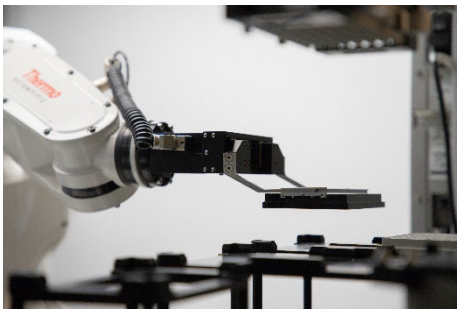
Research Resource Identifier (RRID): SCR_025390

II. How we help

The NBCC provides full support for your screening needs. Our service starts with an initial consultation to identify the best and most cost-effective approach to your project. Our services include assay design, pilot screens, quality control, automated screening, bioinformatics support and assistance with grant and publication writing related to the services we offer.

III. Core instrumentation



The NBCC HTS has two integrated automation systems (Themo F7 and Dim4) that enable multi-day in vitro and cell-based assays with the push of a button. These systems use robotic arms (Spinnaker, F7 and VAL) to move the labware between on-deck instruments such as liquid handlers, washers, incubators and readers. The liquid handlers include those that dispense through tips (Beckman i7 and NXp), acoustic transfer (Beckman

Echo 525 and 555), and bulk dispensing (Thermo Multidrop). Our readers provide end-point standard readouts (absorbance, fluourescence, luminescence, low resolution imaging). All our instruments are available to users as stand-alone equipment.

In addition to these screening instruments, we have an Olink Signature S100 that enables biomarker protein discovery (see applications below).

IV. Screening libraries

A. Chemical libraries

We provide access to chemical libraries for high-throughput screening. We offer full-drug screening of >100,00 drug-like compounds (e.g. Chembridge, Maybridge) or more focused screening using our smaller bioactive libraries (>15,000 compounds many of which are either FDA-approved or tested in clinical trials) or our natural compound library (~4000, most plant-sourced, bioactive).

B. Genome-wide siRNA libraries

We provide access to genome-wide siRNA libraries for human (19,520 gene targets) and mouse (16,640 gene targets). These pooled libraries (4 siRNA in each pool) can be screened in their entirety or as a targeted sub-set (e.g. druggable, kinome).

V. Applications

A. Olink protein biomarker discovery

Olink® Certified Service Provider

Olink offers high-quality protein biomarker discovery based on a flexible and scalable proximity extension analysis (PEA) technology platform which enables multiplexed biomarker analysis. Olink's solutions require less than 2 uL of sample, while maintaining exceptional levels of specificity, sensitivity and dynamic range. As a certified Olink Service Provider, we provide protein biomarker screens on 15 human and 2 mouse Olink Target 96 & Target 48 panels, Flex (15-21 chosen biomarkers) and Focus (custom design) panels.

B. High-throughput Screening

We assist with a wide variety of screening applications. We specialize in automating assays to handle large number of samples and/or treatments. Manual procedures can also be automated to increase accuracy, reduce human error, and avoid repetitive strain injuries. We offer the following screening applications:

Enzymatic Assays: These assays are *in vitro* screens for enzymatic activity that are used to identify enzyme inhibitors or enhancers from chemical libraries.

2D and 3D Cell Culture Assays: Integrated automated screens can be performed *in vivo* (bacterial, yeast, mammalian 2D or 3D cultures) with simple readouts such as cell viability or cytotoxicity or with more complex endpoints that involve high-content imaging. These

screens are often performed under different treatment conditions including using chemical libraries or genome-wide siRNA libraries.

Enzyme-linked immunosorbent assay (ELISA): ELISAs measure specific proteins in a complex mixture and are typically employed to measure antibodies or antigens. For COVID-19, we have validated assays to detect IgG, IgM and IgA antibodies against the spike trimer, spike RBD, and nucleocapsid protein. We also offer a surrogate neutralization ELISA (snELISA) to identify antibodies with neutralizing potential.

Homogenous Assays: These are simplified one-step assays where reagents are added with no wash steps in between. Alpha assays from Revitty typify the type of screening we can offer: 1) AlphaScreen® (ALPHA for Amplified Luminescent Proximity Homogeneous Assay), a bead-based chemistry used to study interactions between molecules in a microplate, 2) AlphaLISA for the detection of analytes in biological samples such as cell culture supernatants, cell lysates, serum, and plasma, and 3) AlphaPlex that can detect 3 analytes by using acceptor beads that emit at distinct wavelengths.

SPAR-Seq (Barcoded pooled screens): SPAR-Seq (systematic parallel analysis of endogenous RNA regulation coupled to barcode sequencing) is a multiplexed and quantitative functional genomics screening platform that can be used for gene regulation profiling when coupled to sequencing output. C19-SPAR-Seq multiplexed COVID-19 positive nasopharyngeal swab samples to track the rise and fall of SARS-CoV-2 variants.

LUMIER (LUminescence-based Mammalian IntERactome): LUMIER is a high-throughput automated platform developed in the Wrana lab for identification of novel protein-protein interactions in mammalian cells. In this assay, a Luciferase (LUC)-tagged fusion protein is co-transfected with a FLAG-tagged protein in mammalian cells. The interaction between the two proteins is determined by co-immunoprecipitating the FLAG-tagged protein and detecting the LUC-tagged interactor in the complex by its luciferase activity.

Library management: We store, maintain, and provide access to commercial and custom libraries. These libraries may also be used to cherry pick specific targets for confirmatory dose-response curves or follow-on validation testing.