Molecular discovery by HTS

Northwestern University
High Throughput Analysis Laboratory
(NU-HTA)

Molecular Biosciences

WCAS

NORTHWESTERN UNIVERSITY

CMIDD
Center for Molecular Innovation and Drug Discovery
a catalyst for the integration of chemistry and biology

Robert H. Lurie Comprehensive Cancer Center

Chemistry of Life Processes Institute
Science Without Boundaries
Discoveries Without Limitss

Center for Structural Genomics of Infectious Diseases
CSGID

NORTHWESTERN UNIVERSITY
FEINBERG SCHOOL OF MEDICINE

Mission

- The NU-HTA provides researchers with equipment, advanced technology, and expertise for the development and execution of chemical screening for drug/probe discovery and high throughput biological analysis.

- In collaboration with our sister facilities at UC and UIC, we provide HTS tools to facilitate your small-molecule discovery and the CBC mission

NU-HTA open to academia and industry

Service Mode

- Provide robust automated solution
- Assist experimental design
- Provide access to robotics
- Collaborate in grant application

- Full service: send material, receive data
- Facility use: you run the experiment
- Development: you bring a problem, we help to find an automated solution

Facilities

- Hogan 4140: molecular and cellular screen
- Hogan 4130: cell culture and assay prep
- Class 100 clean room for primary cell screen

Screen works performed on

Protein **Yeast** **Mammalian cells**

E. coli **Insect cells** ***C. elegans***

Liquid handling & plate detection



Assays and screens

- *Biochemical assays*
  - Enzyme assays
  - Protein-ligand binding by thermal shift and label-free
- *Cell-based assays*
  - Reporter (GFP or Luciferase)
  - proliferation, viability, toxicity
  - GPCR, ion channel, transporter
- *Molecular biology protocols*
- *RNAi profiling*

Screen Libraries

- Genomic RNAi : Human, Mouse
- Yeast genomic : 7 collections
- Small molecule : ~70,000 compounds

Unique instrument in Chicago


FLIPR Tetra


nanoliter dispensers


Colony picker

Equipment Available

- Labcyte Echo550 – non-contact nanoliter liquid transfer
- TTP Labtech Mosquito – contact nanoliter liquid transfer
- Beckman Coulter Biomek FX 96 channel – microliter liquid transfer
- Beckman Coulter Biomek FX Span-8 – microliter liquid transfer
- Integra ViaFILL 8 channel dispenser – "GripTip" technology for low dead volume dispensing
- Genetix QPix II XT colony picker – automated colony picking and library replication
- Genemachines HiGro incubator shakers – for 96 and 384 well plates
- Molecular Devices Analyst GT Multimode plate reader – with onboard plate stacker
- BioTek Synergy 4 Multimode plate reader – with onboard dispenser and monochromator
- Perkin Elmer EnSpire multifunction plate reader – with label-free and Alpha Screen
- Cellomics ArrayScan Vti & Robotic Platform – high content cellular imaging
- BioRad 384CFX qPCR System – 384-well Real Time PCR
- IQ5 Real-Time PCR System – 96-well Real Time PCR
- Molecular Devices FLIPR Tetra – fluorescence plate imaging system for fast kinetics
- TTP comPOUND – -20^{o}C chemical and biological storage system

Biological libraries

Yeast Libraries:

- Yeast TAP collection
- Yeast GFP collection
- Yeast GAL-GST library
- Yeast Two Hybrid Array
- Yeast Deletion Libraries
- Yeast Genomic Tiling Collection

RNAi Libraries

- The NU-HTA provides researchers with the latest in whole genome RNAi collections, has obtained the RNAi lentiviral libraries for both human and mouse (currently 120,000 total clones).

Compound Libraries

As a screening resource the NU-HTA houses a number of small molecule compound libraries that include over 2000 FDA approved drugs.

- ChemDiv Structural-Diversity Set (30,000)
- ChemBridge Drug-Like Set (20,000)
- ASDI Library (6800)
- NIH Clinical Collection (400)
- Silverman Collection (custom library)
- Spectrum Collection (2700)
- NCI/DTP Open Chemical Repository (3200)
- Kinase Inhibitor Collection (80)
- CTCMLD collection: diverse compounds (7000)
- Phosphatase Inhibitors (40)

Assay capabilities

- Protein binding assays
  - Fluorescence polarization
  - Fluorescence based thermal shift (FTS)
  - AlphaScreen/AlphaLISA and ELISA
  - Epic label free
- Enzymatic assays (Dehydrogenase, Kinase, Phosphatase, etc)
- Cell-based assays
  - Promoter/reporter assays (GFP, Luciferase)
  - Cell proliferation, viability, cytotoxicity
  - Receptor functional assays
- Fast kinetic assays (Ion channel and GPCR)
- High content Imaging and analysis
- Large scale or complex liquid handling
  - Hit picking and library reformatting
  - Micro qPCR reactions in 384 well format
- Automated colony picking and microbial library replication

Example:
Protein ligand discovery by thermal shift (FTS)

Protein suitable for FTS:

- Non-membrane protein
- No-priori knowledge of protein's function required

Assay format:

- 384-well, 10 uL assay
- 0.5 ~ 2 ug protein/well
- Single or pooled compound screen (3 ~5)

Throughput:

- 10,000 cpds/day with multi compound (cpd) pooled format

Typical screen:

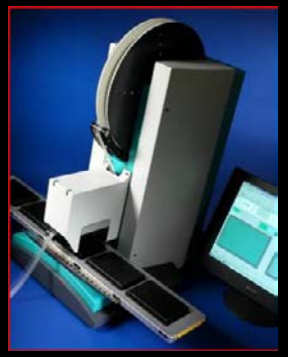
- ~3000 available FDA approved drugs requires ~1 mg protein

HTP small molecule screening by FTS

Protein

Sample prep

Add protein
384-well plate

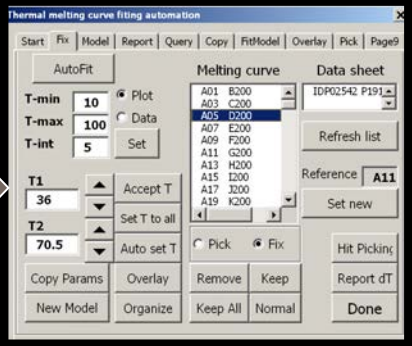


Add 1st screen, uL

Add 2nd screen, nL

Thermal scanning

Data analysis



Report

Ligands and stabilizers