

Resources for High-throughput Screening at MSKCC

Development of Novel Chemical Probe and Therapeutic Regimen

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The MSKCC RNAi Core Facility provides the Tri-Institution with the services of running high-throughput screening (HTS) of a collection of 300K small-molecule compounds. This collection consists of Bioactive & FDA Drugs (9K), Focused Set against Kinase and GPCR (38K), and Diversity Set (260K). We present three representative HTS with the subsets of the collection; these HTS were conducted with biochemical assay with purified proteins or cytotoxicity assay with clonal cell lines or patient-derived cells. The primary screening consists of assay development (not shown), execution, and data analysis including quality assessment and selection of the active compounds (primary hits). The confirmation of the primary hits is dose-response study for prioritization. Our services support research programs at the Tri-Institution to develop novel chemical probe and therapeutic regimen.

Chemical Libraries at MSKCC

Bioactives and FDA Drugs

Library	Supplier	#Compounds
Spectrum Collection	MicroSource Discovery Systems	2,000
LOPAC1280	Sigma-Aldrich	1,280
Prestwick 1280	Prestwick Chemicals	1,280
Tocris	Tocris Bioscience	1,272
Prestwick 1120	Prestwick Chemicals	1,119
Selleck	Selleck Chemicals	993
NH Clinical Collection	Vivace Inc.	727
Seqoia	Seqoia Research Products	202
Signatome	AG Scientific, Cayman, LC Labs, Sigma-Aldrich, ProteinKase de	59
		6,932

Focused Set: Kinase and GPCR

Library	Supplier	#Compounds
SoftFocus Kinase Library	SoftFocus	16,044
Kinase Targeted Library	SFEDCS	6,603
KINASet	ChemBridge Research Labs	3,200
Kinase Library	ChemBridge Research Labs	2,000
Published Kinase Inhibitor Set (PKIS)	GlaxoSmithKline	676
Kinase Set for MSK	Roche	228
		28,751

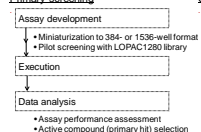
Library	Supplier	#Compounds
GPCR Library	ChemBridge Research Labs	10,080
		10,080

Diversity Set

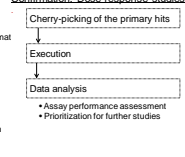
Library	Supplier	#Compounds
Diverse	SFEDCS	74,862
PharmaCophore	ChemBridge Research Labs	54,160
Historic Collection	ChemBridge Research Labs	53,760
ChemDiv	Novartis	44,000
CombiSet	ChemBridge Research Labs	10,000
DIVERSet	ChemBridge Research Labs	10,000
PremiumSet	ChemBridge Research Labs	10,000
Tan Lab Chemical Collection (TLCC)	Derek Tan, Chemistry, MSKCC	464
		257,246

HTS workflow

Primary screening

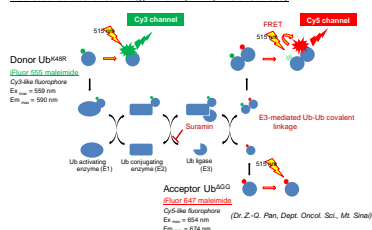


Confirmation: Dose-response studies

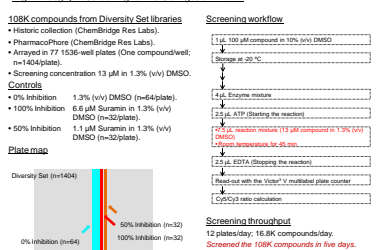


Biochemical High-throughput Screening in 1536-well plate format for E3 Ubiquitin Ligase Inhibitor

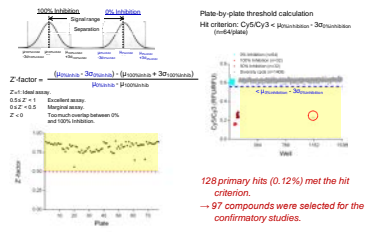
Fluorescence resonance energy transfer (FRET) K48 di-Ub Assay



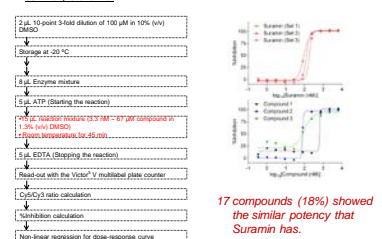
High-throughput Screening for E3 Ub Ligase Inhibitor



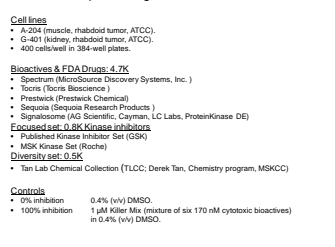
Assay performance assessment



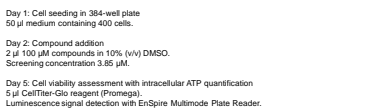
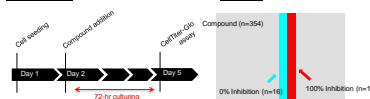
Confirmatory dose-response studies in 384-well plate format



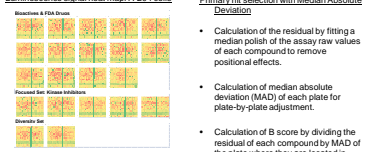
Cell-based High-throughput Screening for Cytotoxic Compounds against Human Rhabdoid Cells



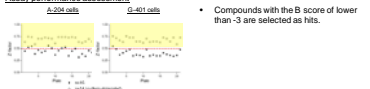
Assay workflow



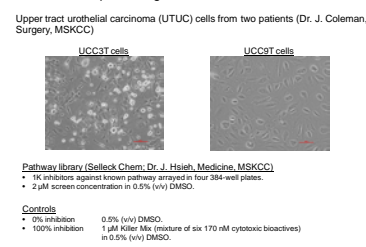
Luminescence signal heat map: A-204 cells



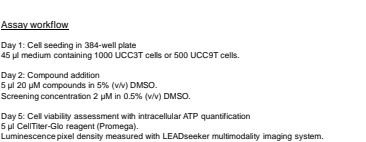
Assay performance assessment



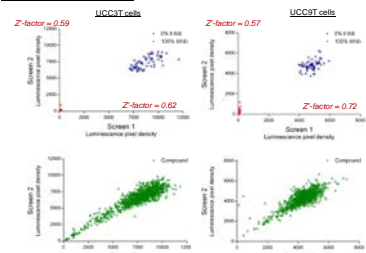
Cell-based High-throughput Screening for Cytotoxic Compounds against Patient-derived Cells



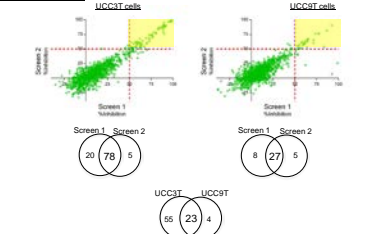
Assay workflow



Assay performance assessment



Primary hit selection



78 inhibitors against UCC3T cells and 27 inhibitors against UCC9T cells reproducibly showed greater than 50% inhibition.

Next step: Confirmation of these primary hits by dose-response studies.