

Integrated HTS platform for innovative drug discovery **Axcelead**

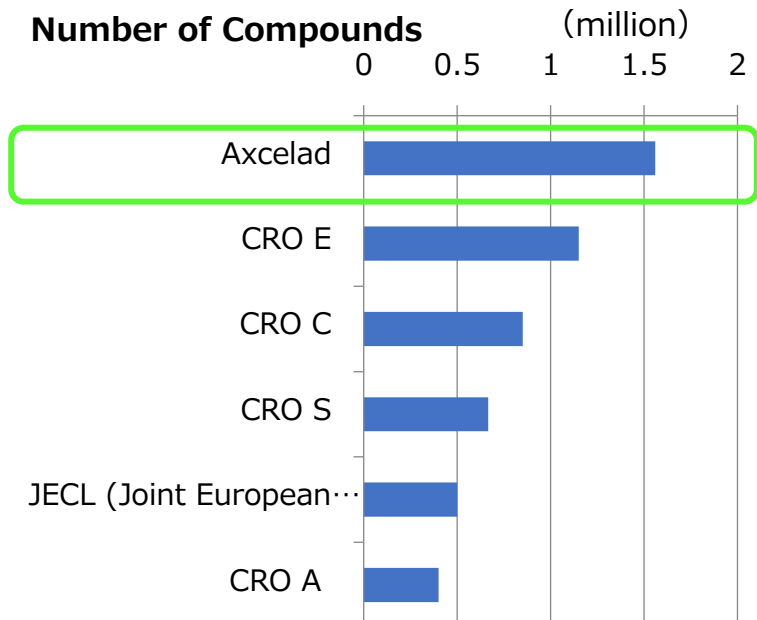
Axcelead Drug Discovery Partners Inc.

The lead-like and diversity compound library

>1,500,000
Compounds

- Purchased compounds : Maximally diverse lead-like cpds, >180 different and selected vendors
 - Inhouse compounds : >600 different programs of small-molecule drug discovery, a rich store of ADMET data
- Library design and synthesis : Novelty, chemical properties, and diversity-oriented

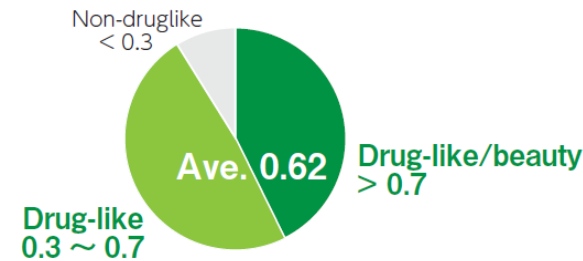
Library Size



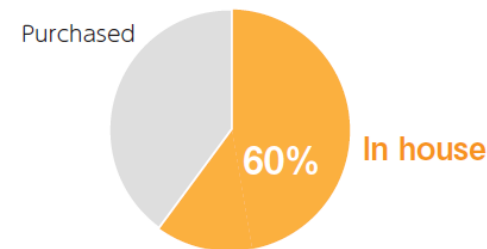
QED: Quantitative Estimate of Drug-likeness
PCA: Principal Component Analysis

Quality

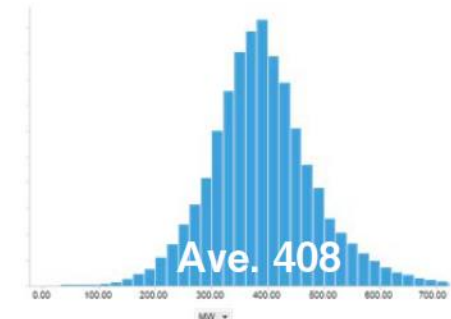
Druglikeness QED: (Chemical beauty)



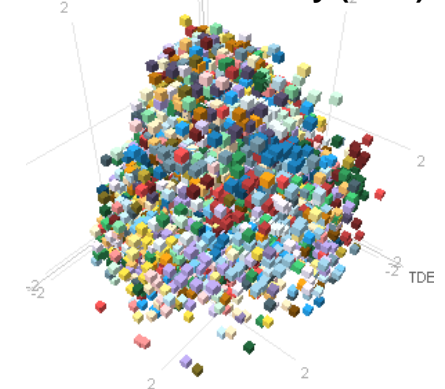
In-house compounds



Molecular Weight



Chemical Diversity (PCA)



Library Sets for HTS

Axcelead libraries >1,500,000 cpds

Diversity,
Chemical Structure
Solubility data,
Cell toxicity data
Activities data

Diversity Library

Single
200K

Diversity Library

- Pilot screening : 2,560
- Clean-A : 10,240
- Clean-B : 44,180
- CNS : 18,900
- sp³rich and chirality rich: 7,500
- Phenotypic Sc. : 22,000
- Fragment (Ro3.5): 11,000
- Extended rule of 5 : 6,400
- Diversity set A : 24,000
- Diversity set B : 18,540
- Core Library (FY21) : 33,000

Pooled
720K

Pool:10 different cpds
per one sample

Diversity Library

- Pilot screening: 32,000
- # 1 : 292,000 (High Priority)
- # 2 : 396,000

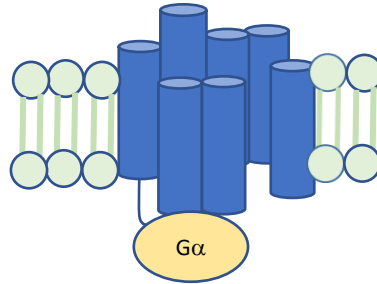
Focused Library
41K

- Target class
 - Kinase : 8,500
 - GPCR : 9,300
 - Protease : 401
 - PPI : 4,100
 - RNA : 6,400
 - RNA splicing (FY21) : 1,280
- Macrocyclic: 6,400
- Natural product: 3,700
- Covalent: 3,800
- Phenotypic Sc.
 - Annotation: 7,000
 - Covalent fragment (FY21): 50

Assay platforms

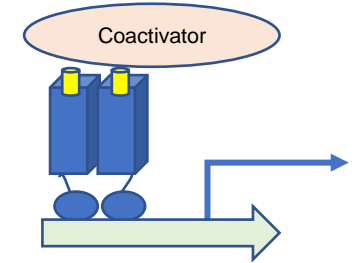
GPCR

cAMP assay
Ca²⁺ flux assay
Reporter gene assay
Arrestin/Internalization assays
Binding assay, Impedance assay



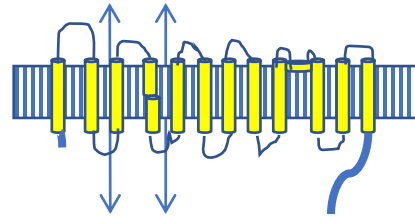
Nuclear receptor

Binding assay
Cofactor recruit assay
Reporter gene assay
Nuclear translocation assay



Ion channel / Transporter

Ion influx assay
Membrane potential
Electrophysiology
Substrate uptake
Binding



PPI (protein-protein interaction)

TR-FRET/Alpha screen assay
ELISA
NanoBit/BRET
Two-hybrid assay
Biophysical assay (e.g. Surface Plasmon Resonance)

Enzyme

Direct assay

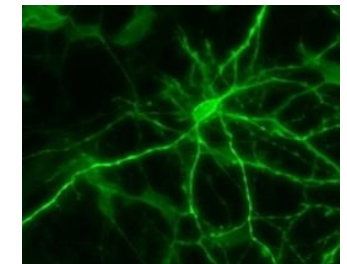
- Absorbance, fluorescence, FRET
- ELISA
- Label-free assay (e.g. HT-MS)

Indirect assay

- Coupling assay (e.g. ATP by luciferase)

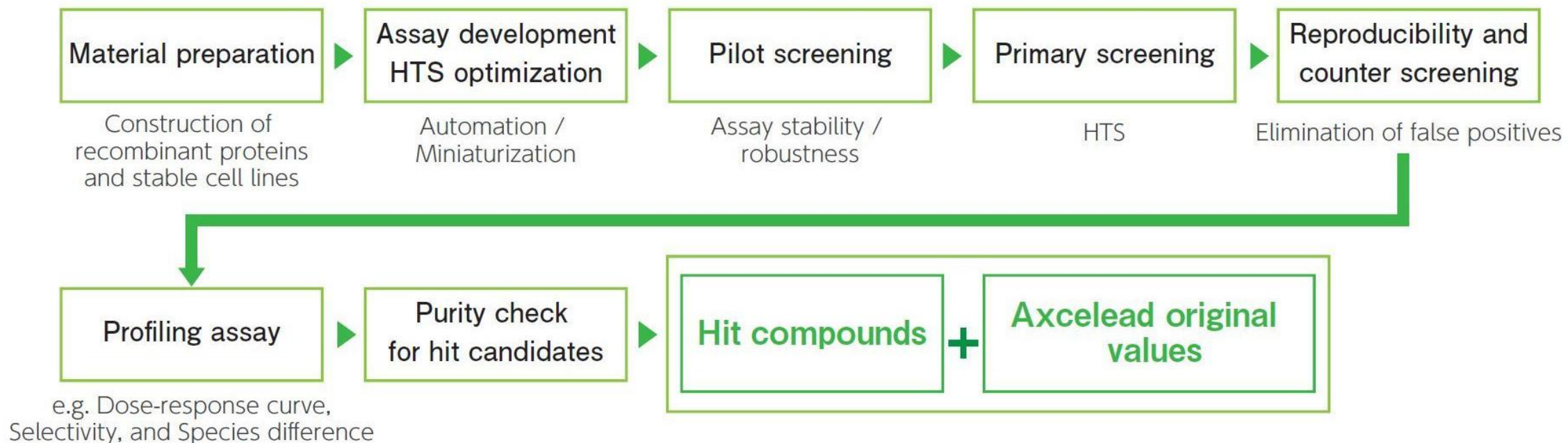
Phenotypic screening

High-content assay
Reporter gene assay
Cell growth
qPCR
CRISPR Cas KO screen etc.



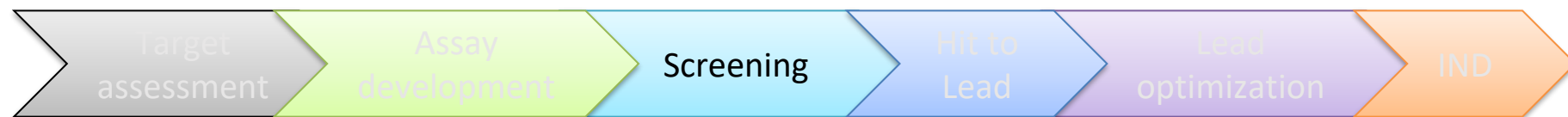
General hit finding process

■ Axcelead comprehensive screening services

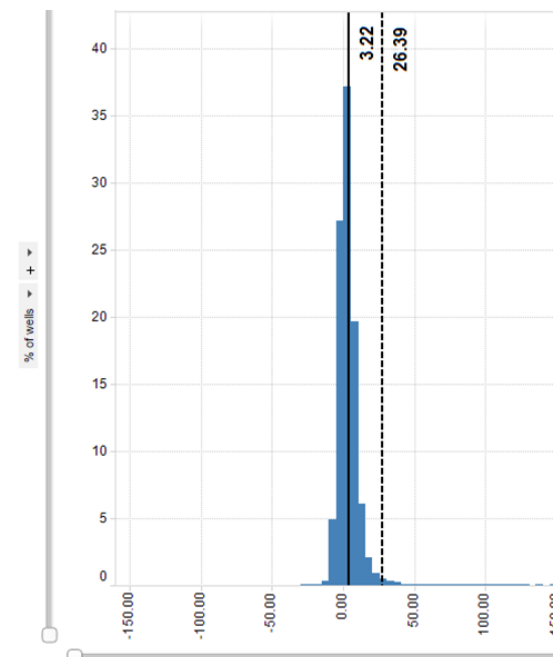
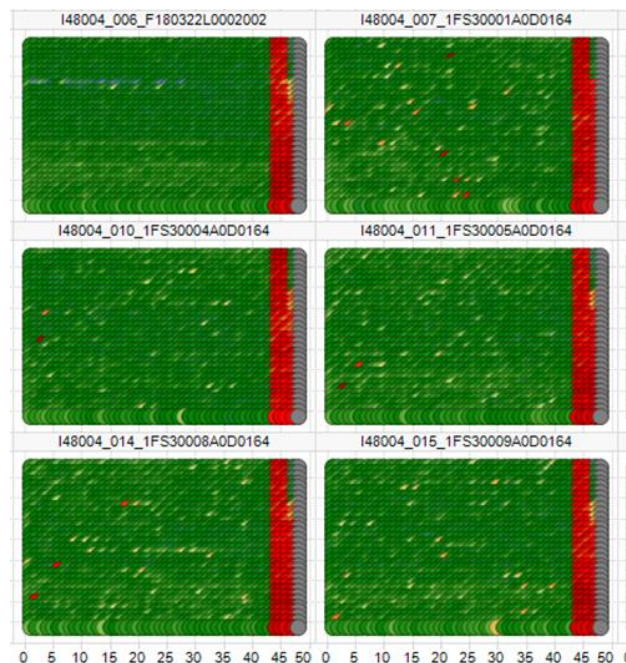


We can also conduct HTS campaigns using each client's assay system.

High throughput Screening (HTS)

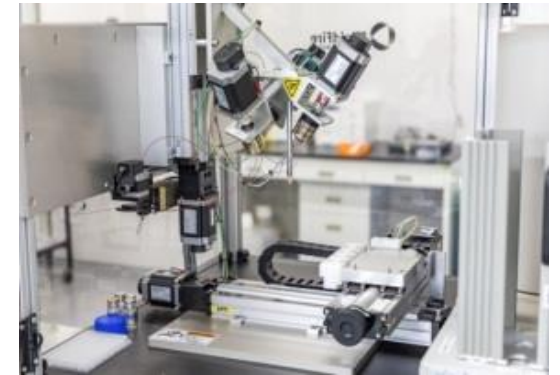


- HTS adaptation
 - Fully automation system
 - Miniaturization (384/1536 well format)
- Pilot screening
 - Feasibility
 - Robustness
- Implementation of HTS
- Data analysis
 - Primary screen
 - Counter assay
 - EC_{50}/IC_{50}
 - Purity of compounds
- Profiling
 - Species difference
 - Selectivity
 - Mode of action etc.



Facilities and capability for general HTS

- Ultra High Throughput Screening capabilities (full automation, 1536-wells format)
- State-of-the-art equipment compatible with diverse assays
 - Acoustic liquid handling system for ensuring high accuracy at low volume
 - Biochemical screening (Fluorescence, Absorbance, Luminescence, qPCR)
 - High-content screening
 - HT-Mass spectrometry screening
 - Electrophysiological screen with HT-autopatch systems
- Data analysis platform to support all screening processes
- Ability to perform HTS under BSL2 conditions
- Ability to perform Radioactive HTS



➤ RapidFire-MS/MS systems
(Agilent)



➤ Fully automated screening system
(FUJIFILM Wako)

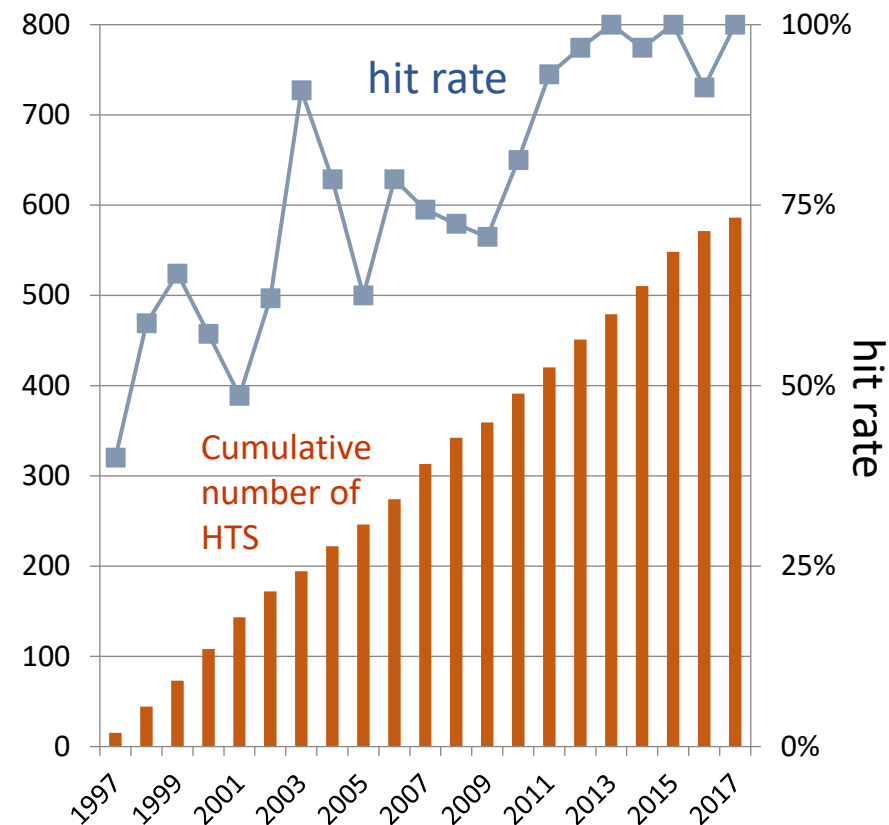
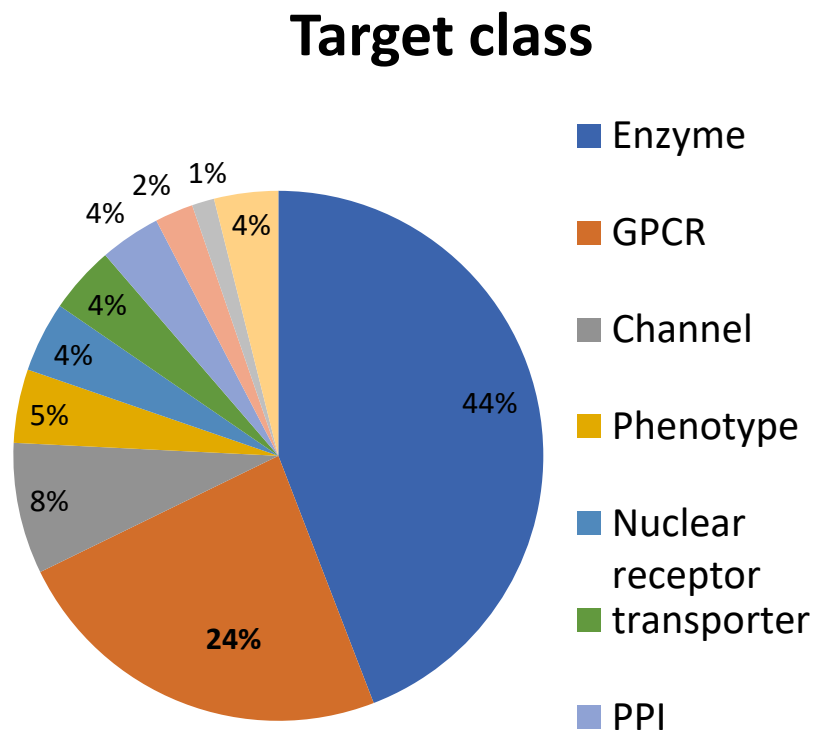


➤ Acoustic droplet dispensing system
(Labcyte)



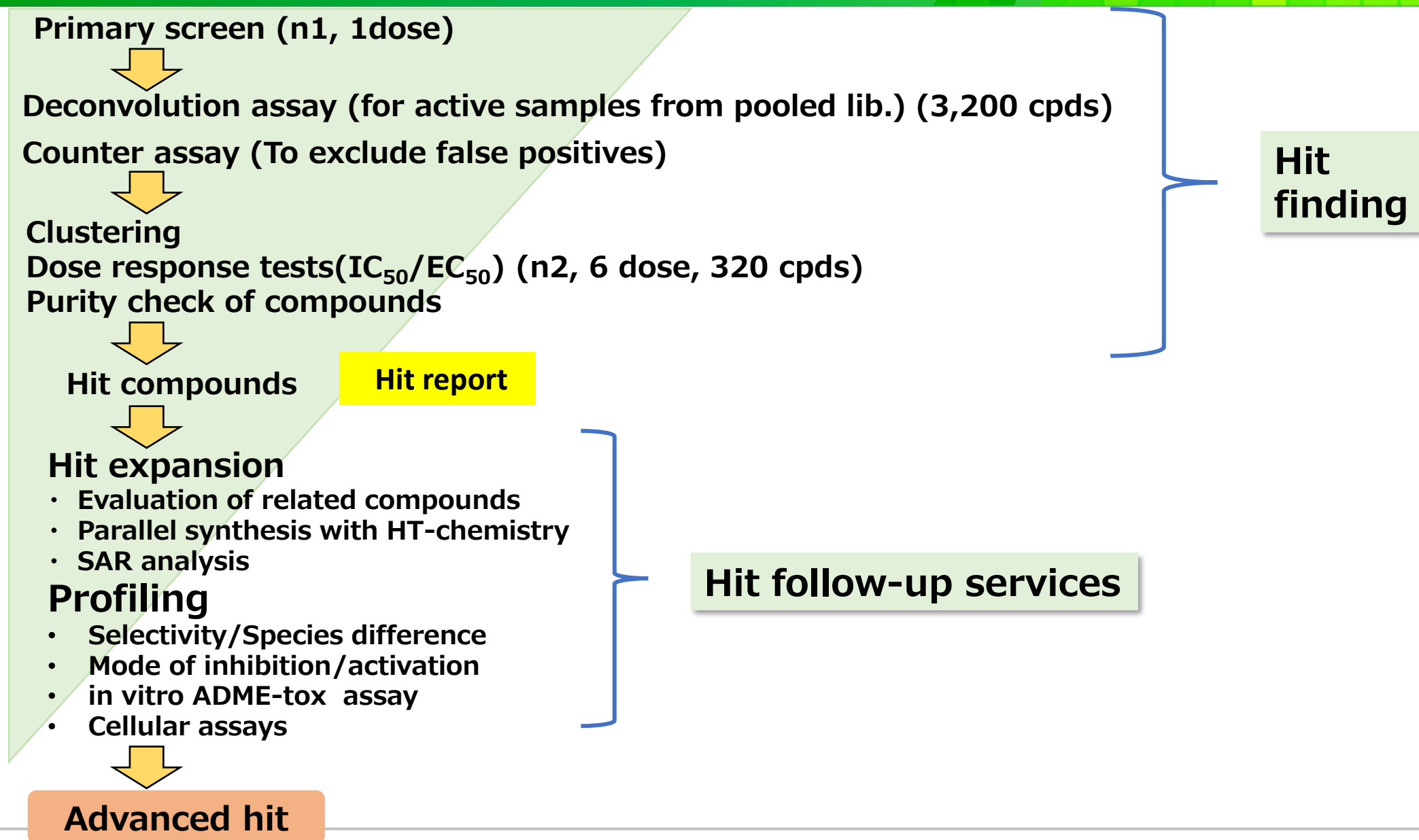
Syncropatch 384 (Nanon)
➤ Autopatch system

HTS track record



➤ Our team has achieved more than 600 HTS campaigns for multiple target classes with high hit rates

General Cascade of Hit Finding



HTS report

Compound No	Cluster ID	Primary assay IC50 (M)	Counter assay IC50 (M)	Primary assay (Graph)	Counter assay (Graph)	Chemical properties				Purity (%)	Notes
						MW	HBD	HBA	HA		
AXL1	1	8.0E-08	>1.0E-05			95.5	kinase A inhibitor
AXL2	1	2.9E-07	>1.0E-05			90.5	kinase A inhibitor
AXL3	1	4.9E-06	>1.0E-05			95.3	kinase A inhibitor
AXL4	2	3.2E-08	>1.0E-05			85.4	promiscuous
AXL5	3	1.2E-06	>1.0E-05			97.2	
AXL6	3	2.5E-06	>1.0E-05			98.1	
AXL7	3	4.2E-06	>1.0E-05			95.4	

- Legacy assay data including annotation (target classes) and cell toxicity etc.
- Chemical properties (QED,HBA/HBD, AlogP, tPSA, Fsp3 etc.)
- Clustering, Comments by medicinal chemists

We offer an HTS report with above information for clients

Capability for Lead Generation/Optimization



- Biochemical assay (potency/selectivity/species for SAR study)
- Mode of action/kinetics analysis and profiling assay
- Cell-based assay (Cellular target engagement, Cellular function etc.)
- Biophysical analysis for target-compound interaction assay
 - AS-MS, TSA, NMR, ITC, SPR, X-ray crystallography



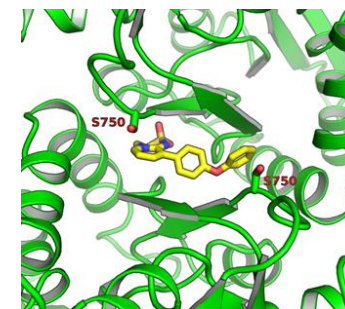
Kinetics assay
with SPR



Thermodynamics
assay with ITC



Electrophysiology assay
with auto patch clamp



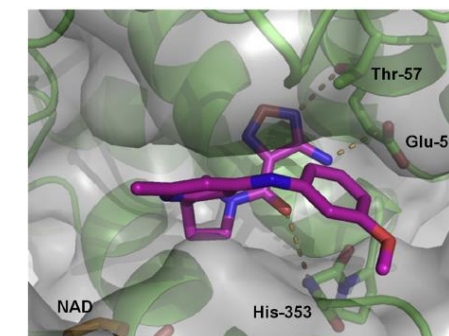
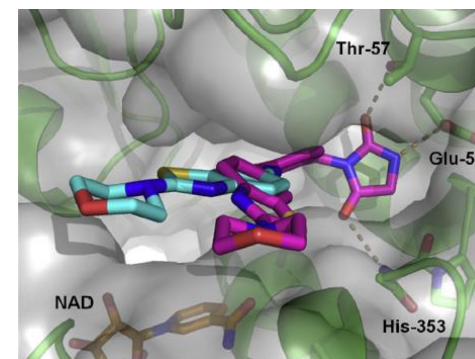
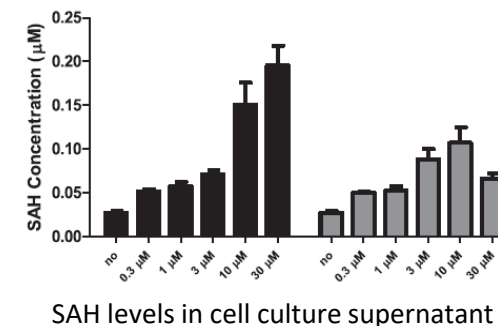
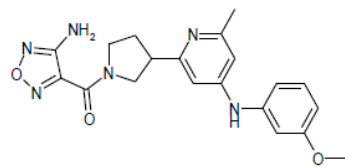
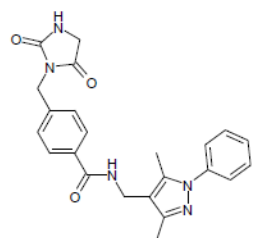
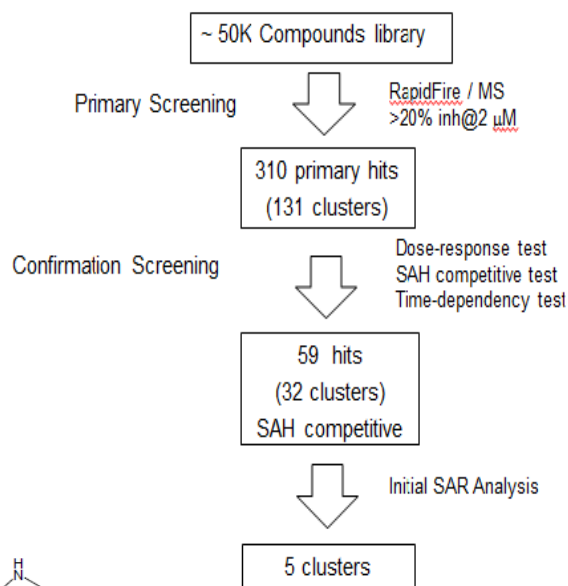
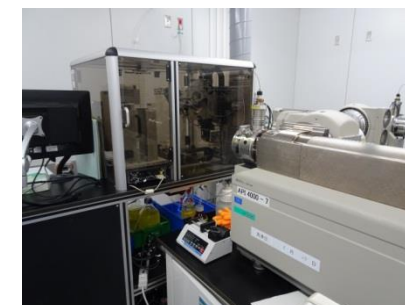
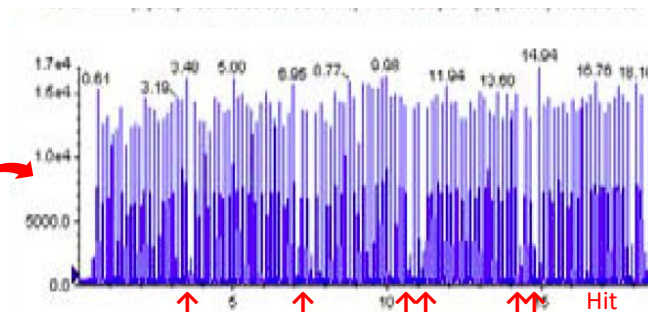
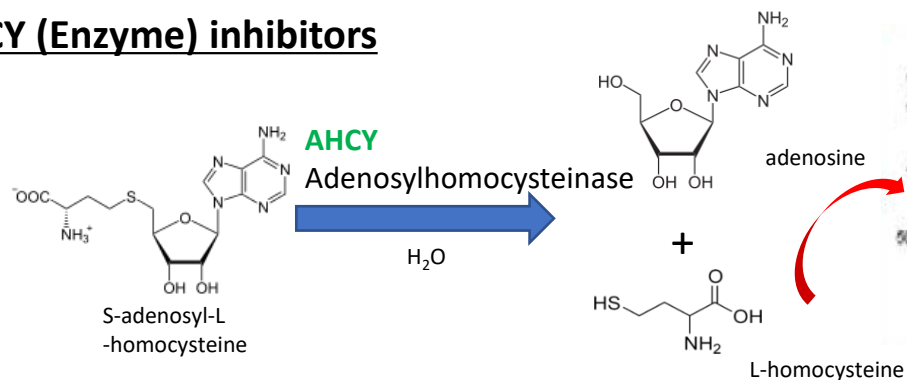
Neuropsychopharm **44** 961–70 (2019)

X-ray crystallography

We provide useful data for drug discovery at the LG / LO stage by using various in vitro assay techniques.

Practical example- hit finding using HT-MS-

AHCY (Enzyme) inhibitors



Crystal structures of AHCY in complex with hit compounds

Biochem Biophys Res Commun. 2017;491(1):1-7

Practical example - GPCR biased ligands-

GPR39 positive allosteric modulators

Primary screening

Library: >600,000 cpds at 3 μ M

hGPR39 cAMP assay (Gs) with EC₂₀ Zn²⁺

Counter assay

Profiling assay

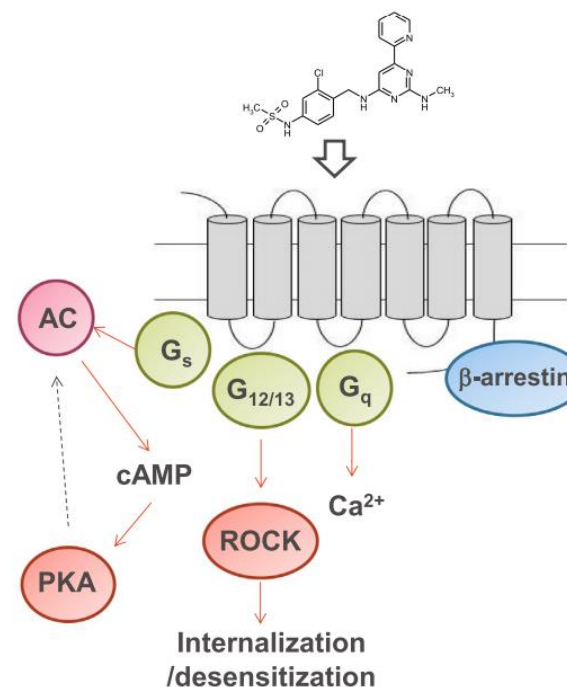
hGPR39 cAMP assay (Gs)

Calcium/IP1 assay (Gq)

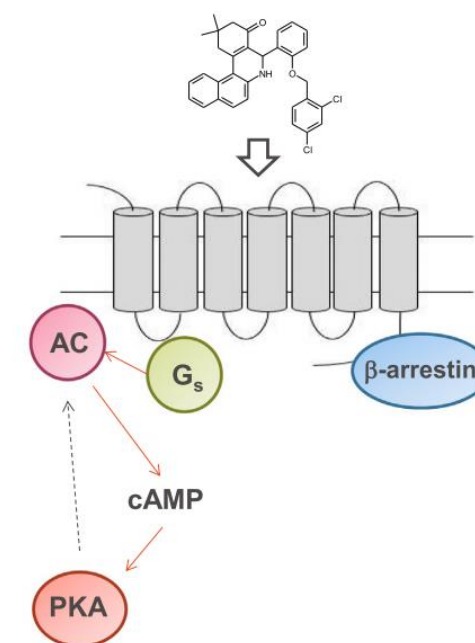
SRE-Luc assay (G12)

Arrestin assay

Gs Biased ligands



Gs biased



Biochemical Pharmacology 140 (2017) 105–114

Practical example-Fragment-Based Approach -

BCL6 inhibitors (PPI inhibitors)

Primary screening

Fragment library (1494 compounds)

200 mM stock in DMSO
<350 Da (ave. 180 Da)

SPR

BIACORE 4000

Single point assay at 1 mM

mtBCL6^{BTB}, wtBCL6^{BTB}, wtBCL6, Neturavidin

➤ 64 compounds (hit rate: 4.3%)

Hit confirmation

Dose response (@ 0.25, 0.5, 1, 2 mM)

➤ 64 compounds

STD-NMR

Bruker 600 MHz w. cryoprobe

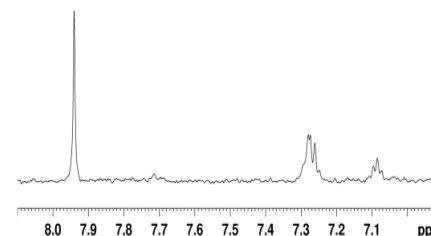
➤ 7 compounds (0.47%)

Competition experiment by SPR

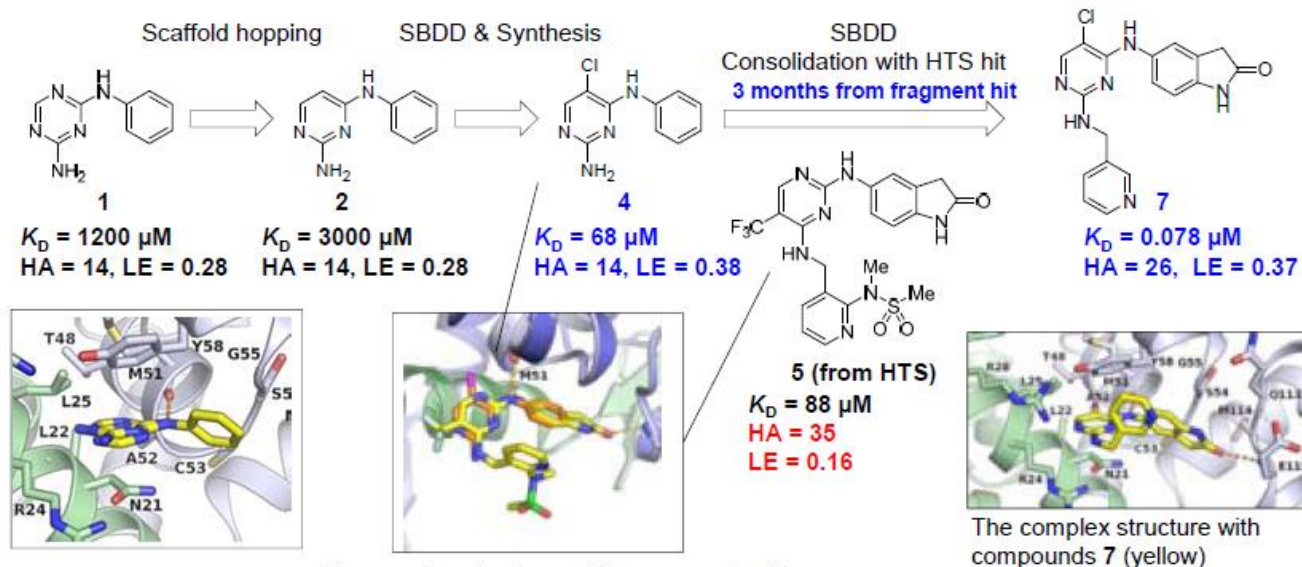
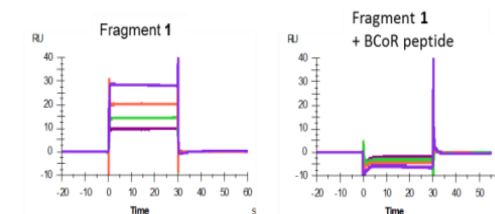
X-tal

➤ 1 compound (0.067%)

STD-NMR



Competition experiment



J Med Chem. 2017 May 25;60(10):4358-4368.

Phenotypic screening

◆ Objectives

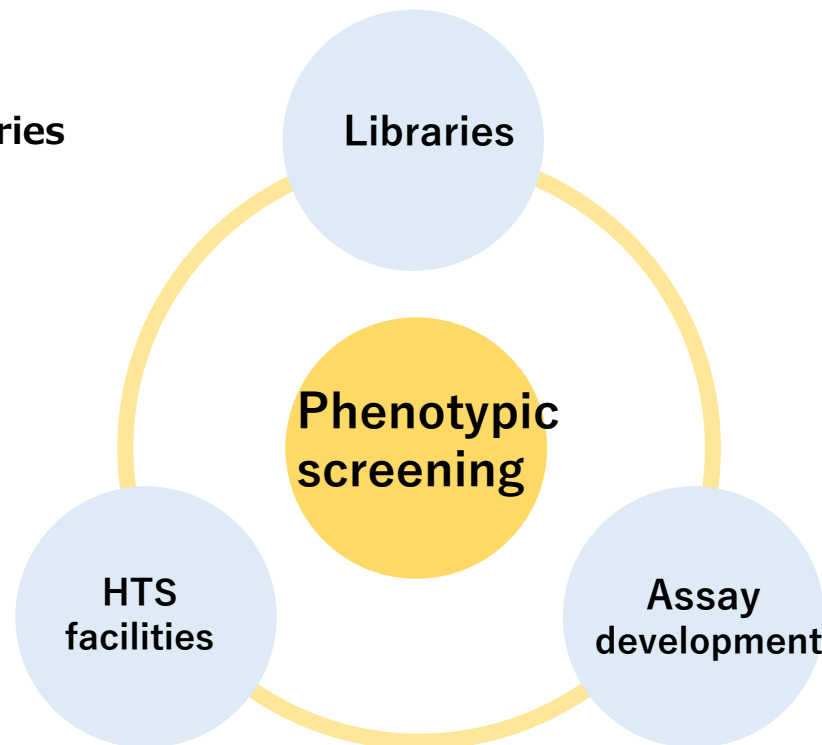
- Hit-Lead finding
- Drug repositioning
- Target discovery

◆ High quality and attractive libraries

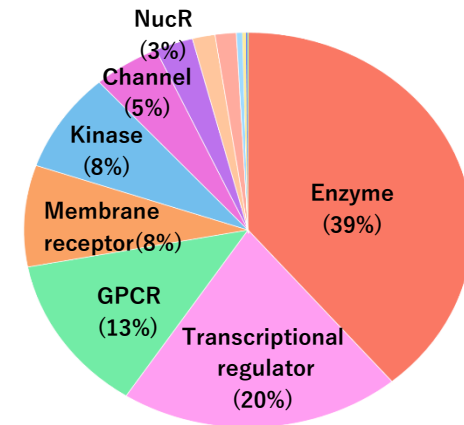
- Diversity
- Biologically anotation
- FDA approved
- Focused

◆ Cutting edge facilities

- Full auto system
- Wide rage of devices
Envision
Incell6000
qRT-PCR system etc.
- BSL2 laboratory



Target profiles of annotated library
(7,000 cpds)



◆ Comprehensive services

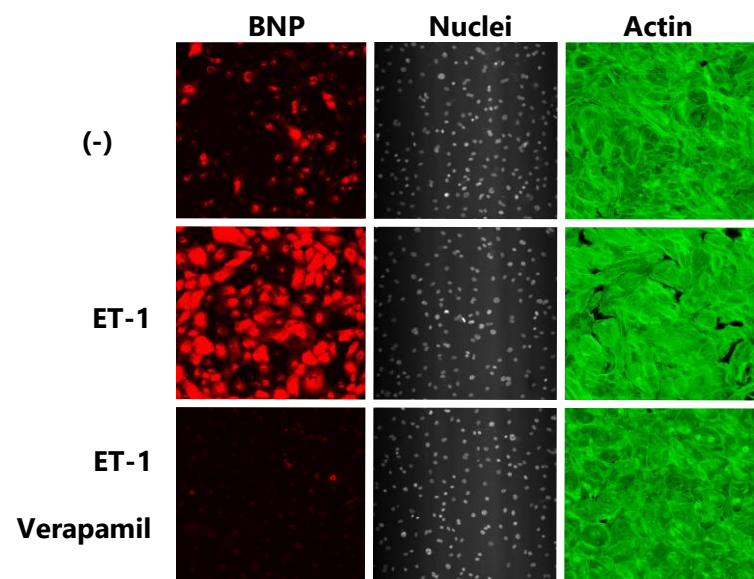
- Cell construction
- Assay platforms
Reporter, RT-PCR, HCA etc.
iPS/Primary cells
CRISPR CAS KO screen
- HTS adaptation

Practical example of HTS using iPS cells

Heart hypertrophy assay with iPS differentiated cardiomyocytes



IN Cell Analyzer 6000(GE Healthcare)



① 1st screening

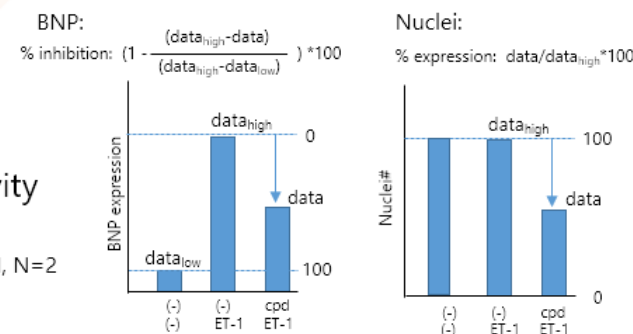
ICC Library:biologically annotated library_3683 compounds, 3uM, N=1
Image analyzing of BNP by INCell 6000

② Reproducibility

ICC 3uM, N=2
ELISA

③ Dose-dependent activity

ICC 0.03,0.1,0.3,1,3,10uM, N=2
ELISA
TaqMan



➤ High content screen using iPS cells resulted in identification of hit compounds including FDA-approved drugs and the related pathways.

Integrated HTS platform

1. Attractive compound library

- Pharmaceutical origin, huge, high-quality and diverse library

2. State-of-the-art infrastructure

- Fully automated screening systems
- Comprehensive platforms covering diverse target classes and phenotypic screens
- A proven track record of more than 600 HTS campaigns for drug discovery

3. High quality and comprehensive services

- Comprehensive services in hit identification including strategy planning, assay development, HTS and profiling
- Hit expansion services including SAR analysis, design and synthesis of related compounds by medicinal chemists
- High-throughput-ADMET profiling services with extensive experience and sophisticated protocols



➤ ***We efficiently offer high-quality hit compounds through our integrated HTS platform***

We are Your Best Partner



Hold hands with You
&
Create Hopeful Future
through Drug Discovery

Axcelead Drug Discovery Partners

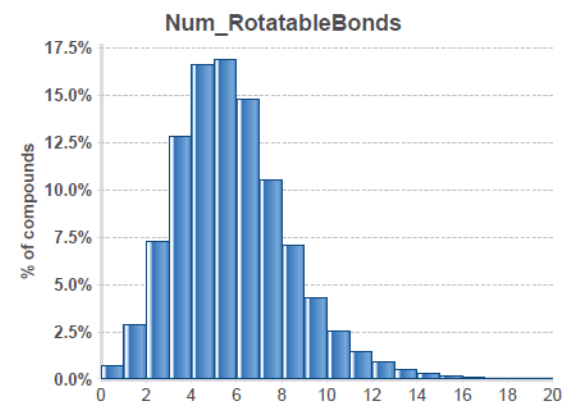
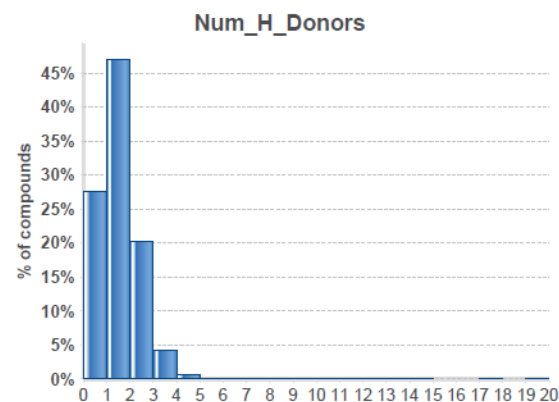
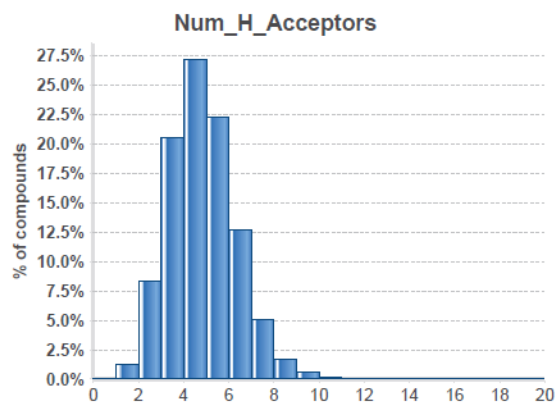
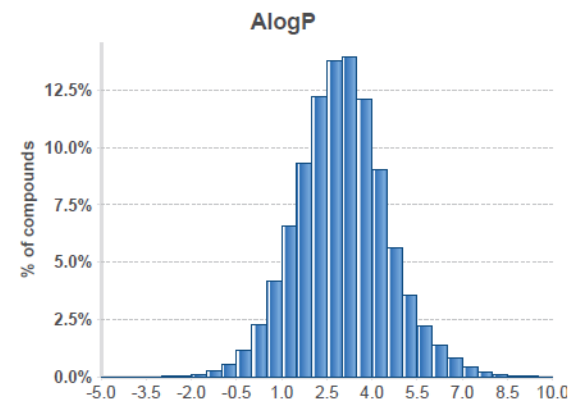
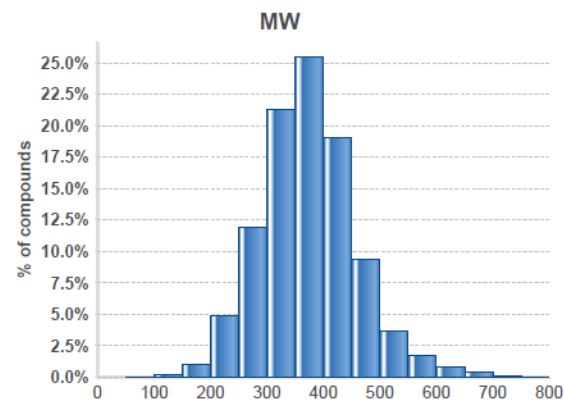
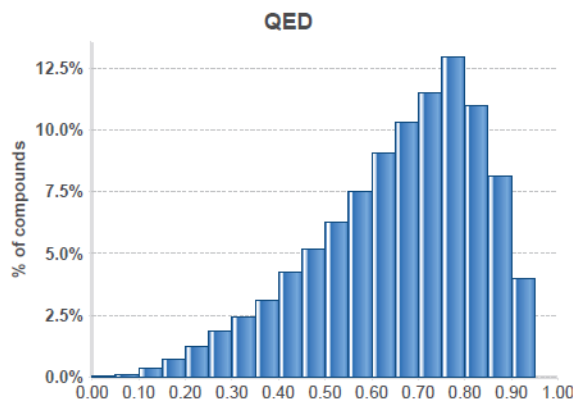
Contact@axcelead.com

Chemical properties of Diversity Library (pooled)



Diversity library (ca.320K)

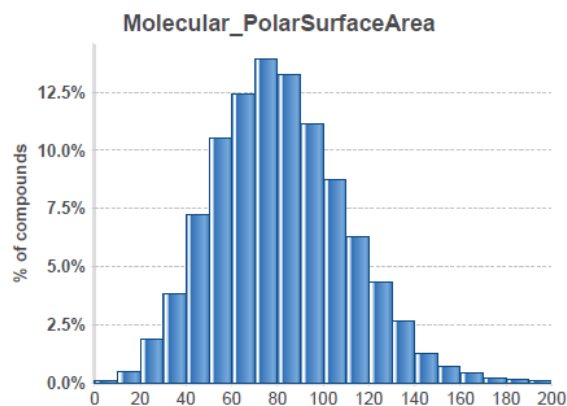
Published at 09/24/20



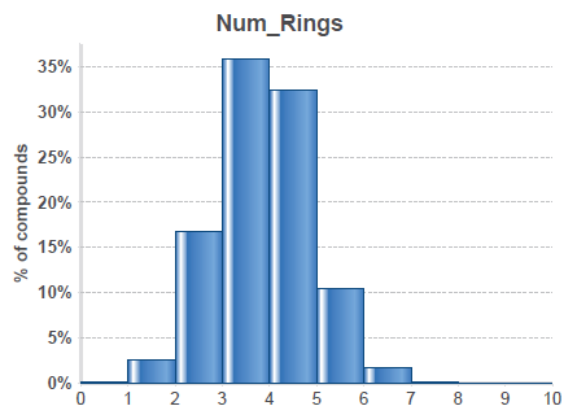
Chemical properties of Diversity Library (pooled)



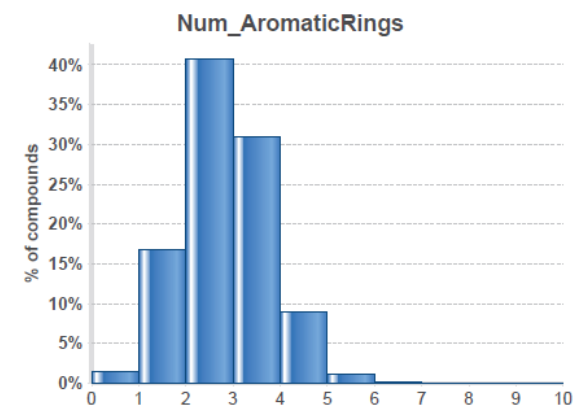
Diversity library (ca.320K)



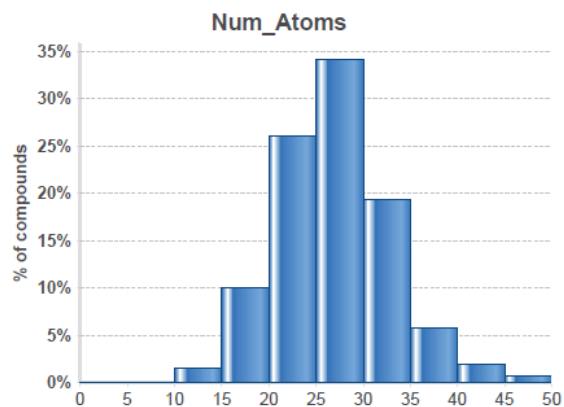
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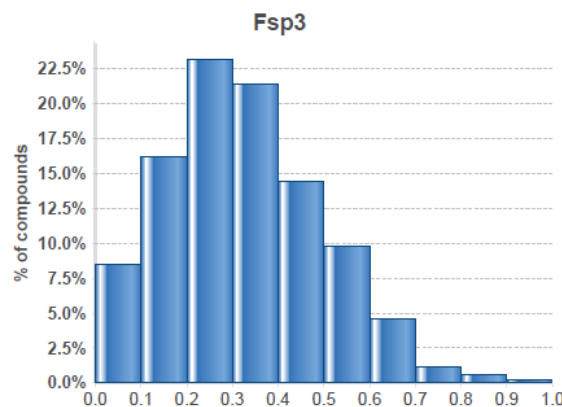
Average: 3.37



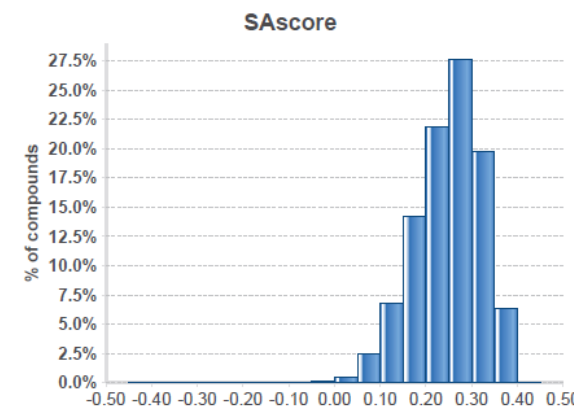
Average: 2.33



Average: 26.5



Average: 0.319



Average: 0.249