

High-Throughput Bioscience Center (HTBC) History and Mission

- Mission: Provide researchers at Stanford and nearby institutions with the ability to run high-throughput chemical, siRNA, cDNA, and high-content screens
 - Created in September, 2003 by Prof. James K. Chen, Department of Chemical & Systems Biology, Stanford University School of Medicine
- Funding provided by:
 - Dept. of CASB (formerly Mol. Pharm.), Sept 2003
 - NIH Shared Instrumentation Grant, April 2004
 - Generous private Donations, Aug 2005 and Dec 2005
 - User Fees
- Funding Opportunities:
 - NIH Roadmap Assay Development (R21) (RFA-RM-07-008)
 - Development of Assays for HTS (R01) (PA-07-320)
 - HTBC can provide letter of support/resources and grant consulting
- Staff:
 - Director-David E. Solow-Cordero, PhD
 - Manage Day-to-Day operations, databases, servers, assay development, robot programming, screen project management, major purchases
 - Research Assistant-Jason Wu
 - Tissue culture, molecular biology, run screens, equipment maintenance, high-content imaging, lab manager

High-Throughput Screening (HTS) at the HTBC

- We provide the ability to rapidly test thousands of molecules in various bioassays.
 - Small Molecule Compound library
 - 130,000 diverse compounds
 - ChemDiv, Chembridge, Specs, Lopac, ChemDiv Kinase, ChemRX, NCI DTP
 - Small interfering RNAs (siRNAs)
 - 21,000 genes
 - siARRAY whole human genome siRNA library from ThermoFisher Scientific (formerly Dharmacon)
 - cDNAs
 - 8000 genes
 - *E. coli* stocks Human ORFeome in Gateway vectors from Open Biosystems
- Assays developed to be robust, inexpensive, and simple to perform.
 - *In vitro* targets:
 - Enzymes, Protein-protein interactions, Ligand-receptor binding, Protein-DNA binding
 - *In vivo* cell based-spectrophotometric:
 - Signal transduction events-Ca⁺⁺, cAMP, reporter gene, proliferation, toxicity
 - High-Content Imaging:
 - Mitosis, protein translocation, cellular morphology, apoptosis

HTS at the HTBC I

- High-Throughput Performance in 384 well microplates:
 - Compound screening:
 - Enzyme/protein/*in vitro* assays: 100,000 assays a week.
 - Compound or siRNA screening:
 - Cell based assays: 75,000 assays a week.
 - High Content Assays: 40,000 assays a week
- Fully automated integrated system:
 - 8, 96 or 384 tip pipettors/pin tools (CaliperLS SciClone ALH 3000)
 - Robotic arms/Stackers for moving microplates (Zymark Twister II)
 - Robotic 198 plate CO₂ Incubator
 - Integrated plate washer (Bio-tek EL405x) and reagent dispenser (Multidrop)
 - Bar Code reader
 - Microplate reader (Molecular Devices AnalystGT)

Available HT Equipment

- Molecular Devices FlexStation II 384 scanning fluorescent microplate reader with integrated fluidic transfers
- Molecular Devices AnalystGT multimode 96/384/1536 plate reader with stackers
- Berthold LB 96 V Luminometer with dual injectors
- Bio-tek EL405x 96/384-well plate washer with Stackers
- Multidrop 96/384-well plate reagent/cell dispenser
- Matrix WellMate--96/384-well plate reagent/cell dispenser with Stackers
- Velocity11 VPrep Pipetting Station (96 disposable tip) and PlateLoc (plate heat-sealer) with BenchCel Stackers
- Qiagen BioRobot3000--automated 96-well DNA minipreps
- ImageXpress 5000A--high content epi-fluorescent microplate imager
- Robotic 44 plate CO₂ Incubator (not yet integrated to ImageXpress 5000A)

SciClone ALH3000

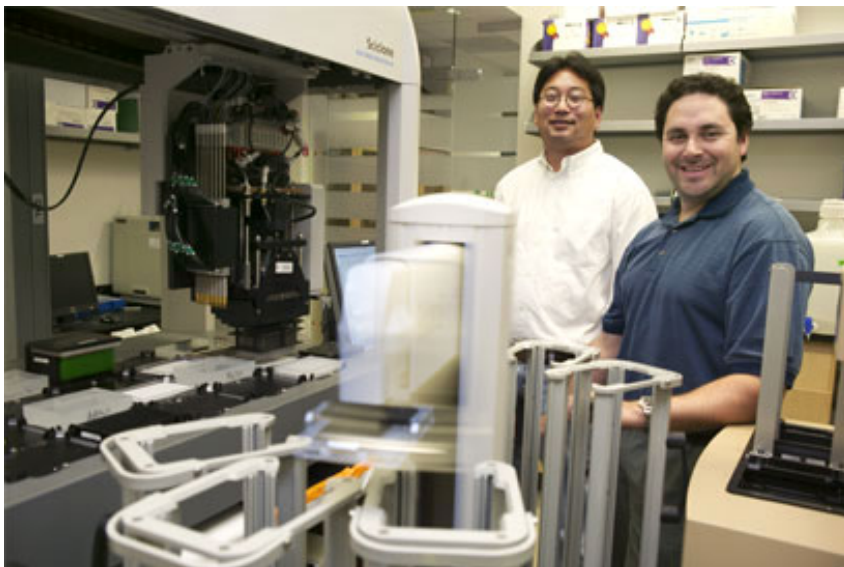


SciClone ALH3000, featuring...

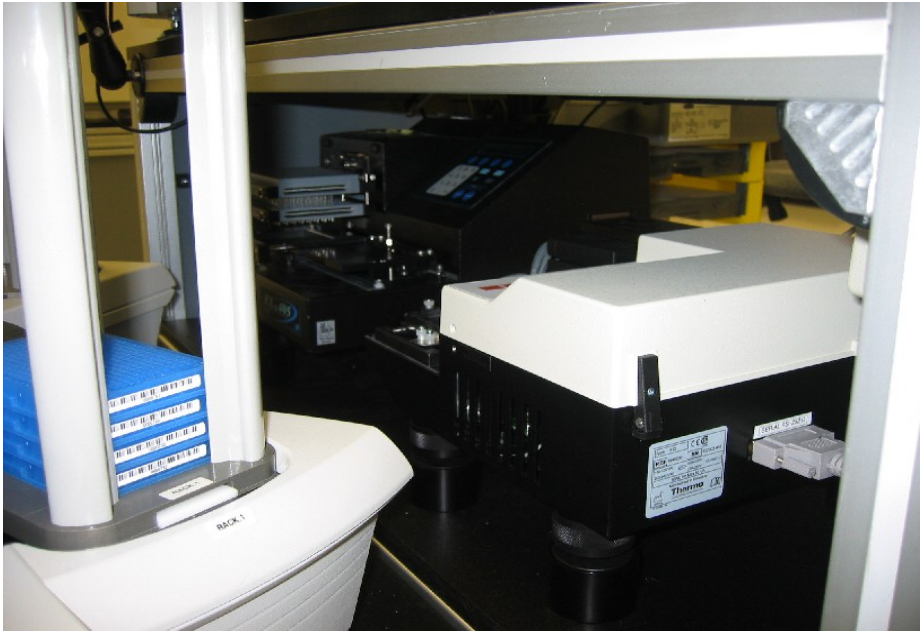
- High Volume Head
 - 96 disposal-tip pipettor (5-200 μ L)
 - Can change tips on the fly
 - Can pick up tips in any array (ie. 1 column or 1 row for dose response assays)
 - Used for 96 to 96 plate replication and 96 to 384 plate reformatting
- Z8 Head
 - 8 independent disposable tip pipettor (5-200 μ L)
 - Primary used for hit picking

SciClone ALH3000

- Low Volume Head
 - Can exchange heads on fly
 - Works with 384 Pin Tool
 - Used to transfer 100 nL of compounds to assay plates
 - 384 Cannula Array
 - 384 fixed tips for low volume (1-25 μ L) reagent addition and mixing
- Other Features
 - Ultrasonic tip washing
 - Recirculating reagent reservoir
 - Filtration station



Liquid Handlers



- Bio-Tek- ELx405 select Plate Washer
 - For 96- or 384-well plates
 - Media exchanges for cell plates
 - ELISA washes
 - Fixing cells for high-content imaging
 - Integrated with robotic system or
 - Stand-alone with stackers
- Titertek Multidrop reagent dispenser
 - For 96- or 384-well plates
 - 5-200 μ L dispensing of reagents or cells

Integrated System I



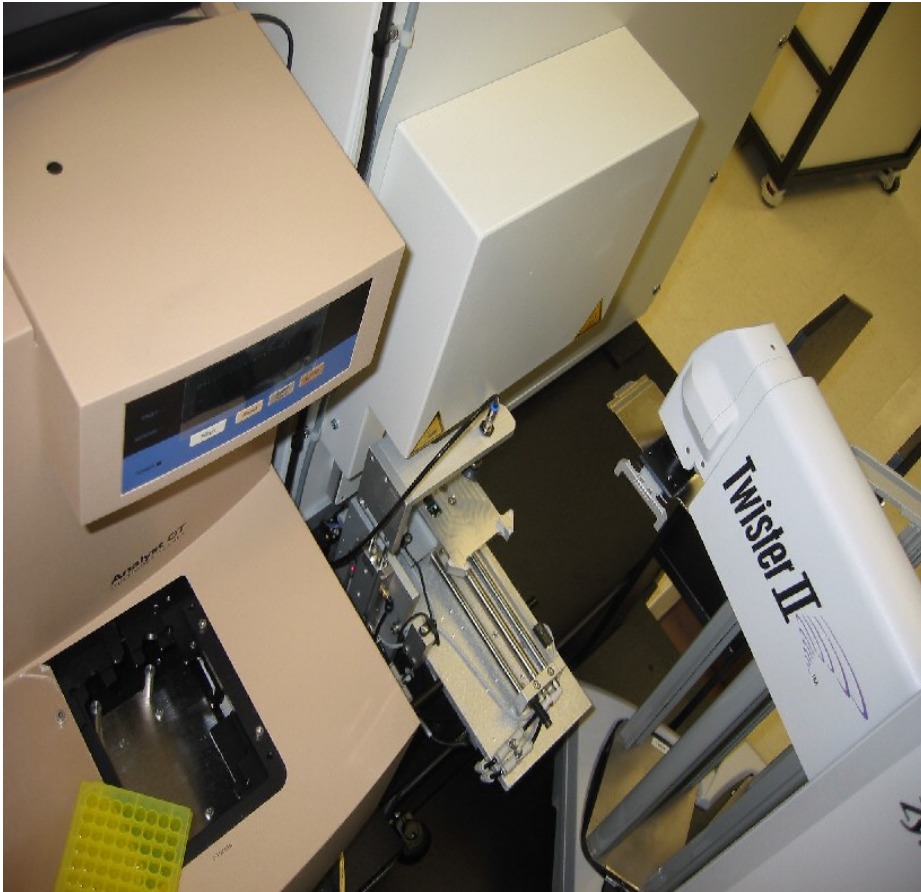
- Twister II robotic arm with stackers integrates entire system to allow fully automated assays
- Can Transport to any other instrument within system:
 - Plates
 - Tips
- Uses the Clara scheduling software

Automated Incubator



- Random access to 198 plates (96- or 384-well)
- Customized plate delivery and de-lidding station

Automated Incubator



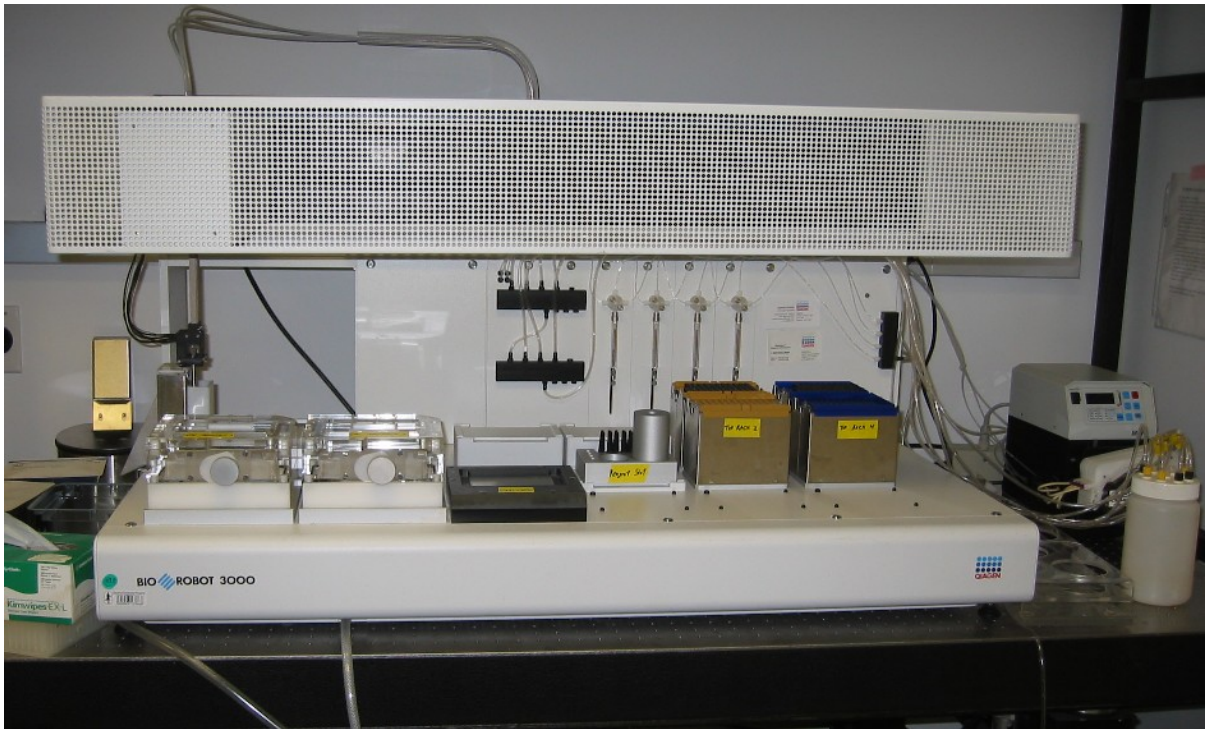
- Twister II robotic arm can take plates from incubator and deliver to plate reader or any other instrument in integrated system.

Wellmate Reagent Dispenser



- Matrix WellMate reagent dispenser
 - For 96- or 384-well plates
 - 1-1000 uL dispensing of reagents or cells
 - Integrated 50 plate Stacker
 - Programmable dispensing (by columns)

Qiagen BioRobot



- Automated RNA/DNA minipreps
 - 2 X 96-well plates in 45 minutes
- 4 Liquid level sensing tips
 - Volume 20-1000 uL
 - Can also act as reagent dispensers for up to 8 different buffers
- Other automated molecular biology protocols
 - Sequence clean up
 - Pooling of samples
 - Reformating of samples, tubes to microplates or vice versa formatting

Velocity11 System



- VPrep liquid handler with 96 disposable tip pipettor (1-200 μ L)
- PlateLoc—automated thermal plate-sealer
- BenchCel Stacker-
 - Automated tip changing
 - Rapid plate replications
 - Rapid siRNA transfections

Molecular Devices Analyst GT



- Multimode Plate Reader
 - Fluorescence Intensity, Fluorescence Polarization, Luminescence-no injectors, Absorbance-UV/Vis, Time-resolved fluorescence resonance energy transfer (TR-FRET), including HTRF
- Features
 - 20 or 40 plate stackers
 - Extremely fast
 - 96/364/1536 well plates
 - Filter based-top or bottom reads
 - Flash and Continuous Lamps
 - Integrated via Twister to allow fully automated screening

Molecular Devices Flexstation II 384

- Fluorescence Plate Reader



- Dual-Monochromometer based
- Can read 6-384 well plates
- Top or bottom reads
 - Standard fluorescent assays
 - MigrationFluoroblock assays
- Internal 8 or 16 tip pipettor allows for measuring kinetic changes
 - Enzyme assays-rapid kinetics
 - GPCRs
 - Ca⁺⁺ flux (FLIPR assays)
 - Ion channels
 - Membrane potential

High-Content Cellular Imaging (in Room 0116)



- ImageXpress 5000A
 - Live cell option-Temp, CO₂, humidity
 - Fluidics-single channel pipettor to image kinetic events
 - Fully automated inverted epifluorescence microscope scans 6- to 384-well plates and records multiple fluorescent images
 - Xenon Lamp/filter based
- Image Analysis Software (3 analysis computers)
 - MetaXpress 2.1
 - ImageXpress (no longer supported)

Databases at the HTBC

- IT Support
 - Using Stanford Windows Server consultant
- Hardware
 - 2 Dell Poweredge2650 servers with external RAIDS and automated autoloader tape backup (LTO-2)
- Databases
 - ImageXpress 5000A images (2.5 MB each, average 20,000 images a day)
 - ORACLE10g Database 3.2 TB RAID5 drive storage (2-3 months of images)
 - HTS Data: ORACLE9i (273 GB RAID5 drive)
- High-Content data analysis:
 - MD/MetaXpress/Axon ImageXpress IXConsole
 - MD Metamorph
 - MatLab
- HTS Data Analysis (for both compound and siRNA screens):
 - MDL ISIS (ChembioAE): Structure viewing
 - MDL Plate Manager: Compound and siRNA plate database
 - MDL Assay Explorer: HT data analysis and storage
 - MDL Report Manager/ISIS for Excel: HT data reporting tool

MDL Assay Explorer

