10 Years of Ultra-high Throughput Screening using HTRF Technology: a Retrospective

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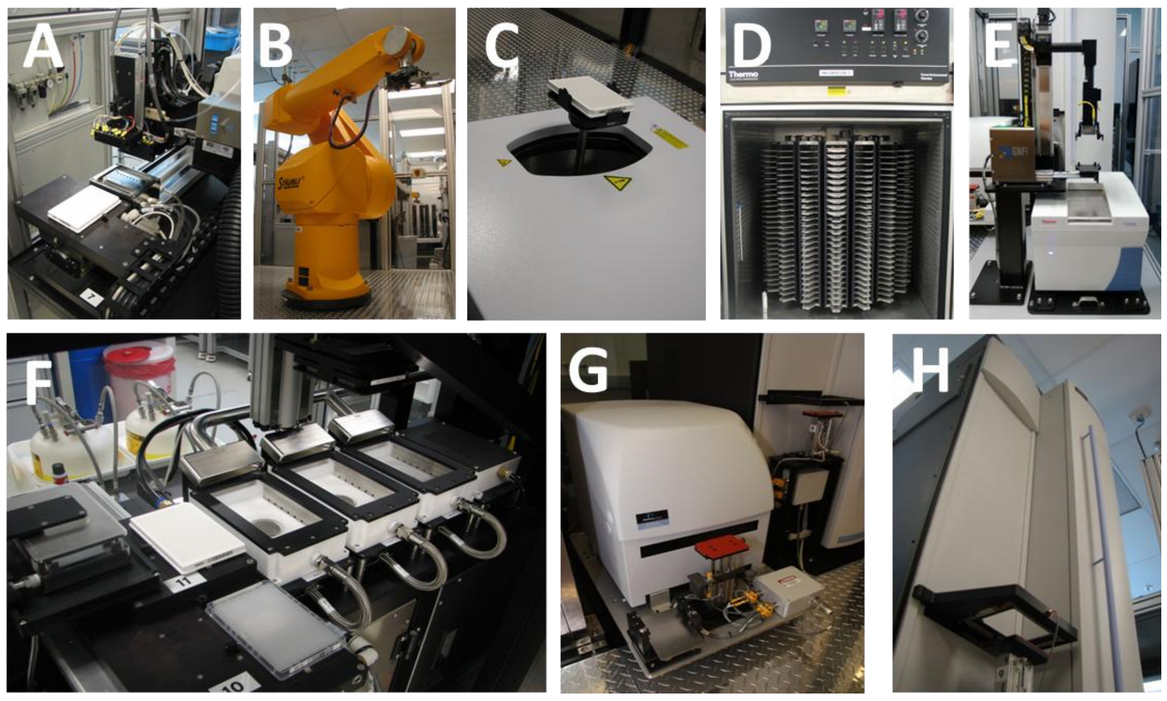
Abstract

Since its inception in 2005, the Scripps Research Institute Molecular Screening Center completed ≈300 ultra-high throughput screening (uHTS) campaigns for both academic and industrial partners. As part of the former Molecular Libraries Probes Production Centers Network (MLPCN, 2005 to 2014), Scripps Florida had the opportunity to participate in a worldwide effort aimed at identifying small molecule probes able to modulate the activity of NIH-approved targets and to share the results with the scientific community through the NCBI's hosted PubChem website.

Here we present a retrospective of the past 10 years using HTRF technology for drug discovery purposes that encompassed >20 primary screens and >80 follow-up assays and contributed to the generation of >5 million datapoints with an average plate Z' of 0.79.

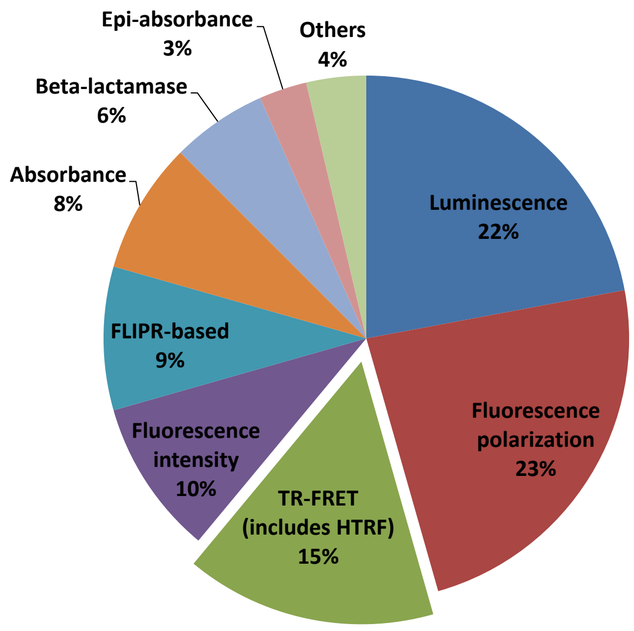
Scripps' uHTS platform

Scripps Florida's screening operations comprises 2 GNF/Kalypsys robotic platforms, one dedicated to compound management and the second, depicted below, that handles biochemical or cell-based assays. All assays used on this platform are miniaturized to the 1,536-well plate format, enabling a throughput of ≈200,000 compounds per day. Access to Scripps Florida's screening platform is possible either through grant co-submission to the National Institutes of Health or through Special Funding Proposals(SFPs). (more information at http://hts.florida.scripps.edu/)



HTRF assay plates are acquired on either the EnVision or the ViewLux readers, both from PerkinElmer (G and H above).

Proportion of HTRF technology

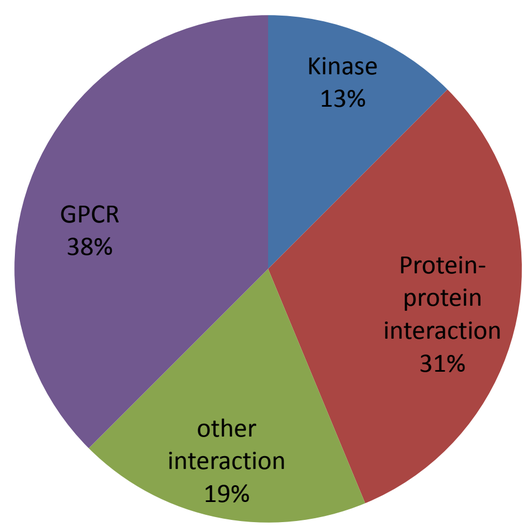


A retrospective analysis of our screening campaign portfolio indicated that TR-FRET represents the 3rd most popular assay technology behind luminescence and fluorescence polarization. Of these TR-FRET assays, ~75% were using CisBio reagents. A total of 5 million datapoints were generated using HTRF technology, with an average Z' of 0.79 ± 0.08, a S/B of 2.84 ± 0.75 and a hit-rate of 0.64 ± 0.39 %.

HTRF assays target types

Protein-protein interactions, together with other kinds of interactions (DNA, RNA, antibody, etc…) constitute half of the target types monitored through HTRF technology. This has been predominantly enabled by the ability to access custom-labeled antibodies. Monitoring of GPCR's activity through second messengers *via* either IP-One or cAMP detection kits represents the second most popular application.

Biochemical and cell-based assays are almost equally represented with 56% and 44%, respectively.



Publicly available HTRF screens

AID	Target Name	Therapeutic Area	Target Class	Assay Type	Assay Provider	Institution	Library Size	Hits	Hit-rate	Average Z'	S/B	CisBio Reagents	Custom labeled Ab?
746	JNK3	Neuro degeneration	Kinase	Biochemical	Phil LoGrasso	Scripps Florida	59,787	366	0.61 %	0.76±0.03	4.14± 1.04	SA-XL 665 - Eu-Ab to b-ATF2	yes
631	PPARg-SRC1	Metabolism	Protein-protein interaction	Biochemical	Patrick Griffin	Scripps Florida	196,180	811	0.41 %	0.73±0.04	2.76± 0.22	Anti-GST EuK	no
1032	PPARg-SRC2	Metabolism	Protein-protein interaction	Biochemical	Patrick Griffin	Scripps Florida	196,179	670	0.34 %	0.70±0.05	2.31± 0.14	Anti-GST EuK	no
731	PPARg-SRC3	Metabolism	Protein-protein interaction	Biochemical	Patrick Griffin	Scripps Florida	196,180	519	0.26 %	0.81±0.02	3.38± 0.13	Anti-GST EuK	no
1899	HCV-Core	Infectious Diseases	Protein-protein interaction	Biochemical	Donny Strosberg	Scripps Florida	302,667	998	0.33 %	0.78±0.07	2.43± 0.74	Anti-GST EuK	no
2300	NR2E3	Retinal degeneration	Protein-protein interaction	Biochemical	Konstantin Petrukhin	Columbia University	315,002	380	0.12 %	0.89±0.01	2.45± 0.12	Eu(K)-antiGST; Streptavidin -D2	no
485270	OX1R	Addiction	GPCR	Cell-based	Patricia McDonald	Scripps Florida	326,028	5,748	1.76 %	0.75±0.09	1.66± 0.21	IP-One Tb Kit	no
624416	Pg9 Ab-GP120	Infectious Diseases	Antibody-protein interaction	Biochemical	Michael Caulfield	International Aids Vaccine Initiative	362,564	1,546	0.43 %	0.94±0.03	3.17± 0.16	SA-XL665; custom-labeled PG9 Eu-conjugated antibody	yes
720596	PrPc	Infectious Diseases	Prion	Cell-based	Corinne Lasmezas	Scripps Florida	369,939	1,596	0.43 %	0.80±0.04	1.91± 0.06	Terbium & d2 custom labeled antibodies	yes
743269	LEDGF p75 DNA	Infectious Diseases	Integrase	Biochemical	Mamuka Kvaratskhelia	Ohio State University	369,939	2,353	0.64 %	0.91±0.03	3.96± 0.83	Eu-SA	no

The table above lists the different MLPCN-sponsored HTRF screening campaigns that were made publicly available through the PubChem website.

To access a specific screen's results and assay protocols, use the following link: https://www.ncbi.nlm.nih.gov/pcassay?term=XXX, where XXX needs to be replaced by the Assay Identifier (AID) number indicated above.

Publications

In pursuit of synthetic modulators for the orphan retina-specific nuclear receptor NR2E3. Qin Q, Knapinska A, Dobri N, Madoux F, Chase P, Hodder P, Petrukhin K. J Ocul Pharmacol Ther. 2013 Apr;29(3):298-309

Comparison of miniaturized time-resolved fluorescence resonance energy transfer and enzyme-coupled luciferase high-throughput screening assays to discover inhibitors of Rho-kinase II (ROCK-II). Schröter T, Minond D, Weiser A, Dao C, Habel J, Spicer T, Chase P, Baillargeon P, Scampavia L, Schürer S, Chung C, Mader C, Southern M, Tsinoremas N, LoGrasso P, Hodder P. J Biomol Screen. 2008 Jan;13(1):17-28.

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Unique drug screening approach for prion diseases identifies tacrolimus and astemizole as antiprion agents. Karapetyan YE, Sferrazza GF, Zhou M, Ottenberg G, Spicer T, Chase P, Fallahi M, Hodder P, Weissmann C, Lasmézas CI.. Proc Natl Acad Sci U S A. 2013 Apr 23;110(17):7044-9.

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