HIGH THROUGHPUT AND HIGH CONTENT SCREENING - PROJECTS AND CAPABILITIES

LOKAVYA MEENAKSHI KURUP, VANDANA G PAWAR, CHANDAN MITHRA, DHRUV RAINA, BALAJI RAMALINGAM, SHAHABUDDIN M S, VARADHARAJAN S, SATYAJIT MAYOR AND APURVA SARIN

National Centre for Biological Sciences (TIFR), Bellary Road, Bangalore 560 065, India

VISION

Expand the scope & objectives of academic sciences, foster collaborations in multi disciplinary contexts, bridge gap between basic and applied research.

INTRODUCTION

High throughput screening allows for the rapid identification of active compounds or genes which regulate a particular cellular pathway or target, especially in novel situations where literature is unable to provide sufficient information to zero in on a practical number of active entities.

The screening facility at NCBS was initially set up to identify genes involved in endocytosis in Drosophila S2R+ cells, and over the last couple of years, has evolved to cater to a broader range of applications. Currently, the screening facility houses its own dsRNA libraries, as well as offers the space and equipments necessary for carrying out small molecule chemical interaction screens.

SCREENING FACILITY – CORE STRENGTH

CELL BIOLOGY UNIT

The advent of RNAi-mediated knockdown has accelerated discovery biology programs in basic and translational life science research. Equipped with cutting-edge detection technologies and an integrated robotics platform, the Screening Unit provides the infrastructure and technical expertise to support genome-wide siRNA-based screens. This approach will enable our collaborators to identify novel signalling networks with relevance to normal and disease-associated states. With our BSL-2 compliant tissue-culture facility, and access to common TC reagents and equipment, the HTS facility provides infrastructure for users to culture their cells within the lab itself.

CHEMICAL BIOLOGY UNIT

Integrating synthetic chemistry with in vitro and cellular biology, the unit investigates novel drug discovery targets through chemical interaction screens or through cell-based assays, in an attempt to bridge the gap between the fundamental biological research conducted both within the Institute and the wider University community.

INFRASTRUCTURE

IMAGING PLATFORMS

THERMO SCIENTIFIC CELLOMICS® ARRAYSCAN® VTI

The Cellomics Arrayscan is a modular high content screening microscope designed for automated fluorescence imaging and quantitative analysis of fixed and live-cells. The instrument at the HTS facility is an epi-fluorescence scope featuring Zeiss optics and a broad spectrum light source. The fully automated design allows for multichannel imaging of nearly any optically-clear/glass bottomed form factor - from 35mm coverslip dishes to 1536 multiwell plates. Our microscope is also equipped with a liquid handling unit for assays where the time between addition of compound and imaging i extremely critical. In addition, we also have the Catalyst Express add-on robotic arm, which can automatically move plates into the microscope, ensuring our scope can be active 24 hours, and reducing the possibility of manual error for time-bound experiments.

Currently, our Arrayscan platform has the following configuration:
Objectives: 20X (NA 0.40), 40X (NA 0.50), 20X (NA 0.80), 40X (NA 0.75), 63X (NA 0.75)
Excitation Filters: 365, 475, 535, 549, 572, 630, 655, 695 nm
Emission Filters: 515, 535, 600, 640, 695, 730 nm
Brightfield Optics: None
Camera: 12-bit

Thermo Scientific Cellomics Arrayscan VTI

NIKON ECLIPSE TiE

Our Eclipse platform is an extremely flexible and fully automatic epi-fluorescence microscope. It is equipped with a 16-bit Cascasde II 512b CCD camera, automated shutter controllers, a motorized stage, fully automated turrets for excitation and emission filters, and also has a hardware-based autofocus system. The entire setup is seated on vibration isolation tables from Newport. Multichannel acquisition of pre-defined positions is fully automatic and extremely rapid, and can be adapted to virtually any form factor. This system also features DIC optics.
Currently, our Nikon has the following configuration:

Objectives: 20X (NA 0.75), 40X (NA 0.95), 60X (NA 1.4)
Excitation Filters: 350, 403, 568, 501, 654
Emission Filters: 475, 535, 610, 710
Brightfield Optics: DIC
Camera: 16-bit EMCCD

Nikon Eclipse TiE

LIQUID HANDLING PLATFORM

TECAN FREEDOM EVO WORKSTATION

For complex liquid handling needs, and to set up high throughput chemical interaction assays, we have the Tecan Freedom Evo platform. This comes with a 200-cm deck, multiple positions for labware, a robotic plate handling arm, and two liquid dispensing pipette heads - one 8-channel flexible liquid handling arm for cherry picking and random formatting with capacities from 100nL to 500uL, and one 96/384 head with automatic changeover between heads for dispensing into multiwell plates. In addition, the workstation is also equipped with a Tecan M1000 Infinite Pro multimode plate reader capable of multichannel absorbance, luminescence, fluorescence readings, as well as polarization readings. All arms are managed parallely, allowing scheduling of liquid handling along with plate logistics to provide efficient use of all resources.

Tecan Freedom Evo

** Information taken from the company website*

IMMUNOASSAY PLATFORM

BIO-PLEX MAGPLEX MULTIPLEX READER

The Bio-Plex MAGPIX multiplex reader is a compact, robust system for magnetic bead–based immunoassays. This multiplex reader is capable of reading assays designed on magnetic xMAP (MagPlex) beads. This can analyse up to 50 analytes per sample. It has a simple and convenient ELISA-like workflow. It provides improved multiplex productivity and is convenient with magnetic bead–based assays. It is also capable of conserving precious resources with an affordable low maintenance system. Performance monitoring during data acquisition alerts the user to performance issues and loads recommended maintenance to resolve issues

Bio-Plex Magplex Multiplex Reader

ASSAY FORMATS

Apart from standard form factors such as 96/384/1536 well plates, the screening facility also houses our custom-designed 300-well slide format for microscopy based screens which have complex assay requirements

ONGOING PROJECTS

CELL BIOLOGY UNIT

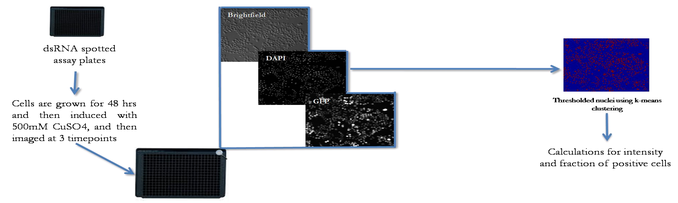
ENRICHED SET MICROSCOPY-BASED RNAI SCREEN FOR GENES INVOLVED IN KINETICS OF MUTANT VAP AGGREGATION

Dr. Girish Ratnaparkhi IISER, Pune

VAP Proteins (VAMP Assoc. Protein) are conserved integral ER proteins implicated in a number of processes, including lipid transport, membrane trafficking and neurotransmitter release. The VAP mutant p58s behaves as a dominant negative mutation by causing aggregation of mVAP, and recruiting wtVAP into those aggregates. This loss-of-function of VAP is loosely associated with motor-neuron disease.

Stably transfected metallothionein-VAP-GFP(wt),VAP-GFP(p58s) and PRM-GFP Drosophila cells are cultured, and RNAi is carried out on them using a 2-day protocol on a 384 well glass bottom format. Metallothionein promoter is induced using copper sulfate and images are acquired at two time-points, 24 and 36 hours, to monitor mVAP aggregation kinetics using the Cellomics Arrayscan Platform. Images are then segmented and cells are detected using custom MATLAB routines that segment out nuclei, and then estimate fraction of cells with aggregates, and number of GFP-aggregates per cell. Primary hits are picked by selecting genes which significantly ($p>0.05$) perturb the fraction of cells containing aggregates compared to controls.

Pilot screen and Phase 1 was successfully completed on p58s,PRM and VAP lines for about 520 genes(including secondary screen). A plate layout of 114 genes (in triplicates) was followed, each repeated at two different time points in 384 well format. Phase 2 for another 600 genes is partly completed with analysis still underway.



SCREEN AGAINST MOLECULAR PLAYERS OF DDR PATHWAY TO IDENTIFY FUNCTIONAL SMALL MOLECULE INHIBITORS OF DNA REPAIR IN CANCER THERAPY

CCBT, Bangalore

The treatment for cancer, chemical or radiation, attempts to target the DNA of cancer cells and inflict double stranded breaks, damaging the genome enough to drive the cell into apoptosis. DNA Damage Response, being one of the most meticulous pathways in a cell, recruits repair molecules that diligently endeavor to rescue the damaged cancer cells, compromising the effect of treatment.
Implicated hits from the small molecule screen are tested, with the help of High Content Imaging, for their inhibitory activity against multiple yardstick molecular players of the DNA Damage Response pathways, after inducing double stranded DNA breaks by employing a combination of irradiation and chemical treatments. Cell-based assays are being standardized to optimize drug dose response on cancer cells, in which the extent of DNA damage is dictated by formation of nuclear foci. At present, assay miniaturization and optimization into multi-well format is also underway.

SCREEN FOR CHEMICAL EFFECTORS OF AUTOPHAGOSOME FORMATION USING HIGH CONTENT ANALYSIS

Dr. Varadharajan Sundaramurthy NCBS,Bangalore

Microtubule-associated protein 1A/1B-light chain 3 (LC3) which has been identified as the specific marker for autophageal compartments where LC3 II, a post translational variant of LC3 I, localises to the membrane of these compartments. Cell lines stably transfected with GFP bound LC3 II are used to follow dynamics of autophagosome formation(in this case HeLa BAC cells) Experimental optimization on 384 well format and customization of imaging parameters for a chemical screen that follows perturbations in autophagosome formation relative to untreated controls is currently ongoing.

MANUAL AND AUTOMATED IMAGING

Radiant Research,Bangalore

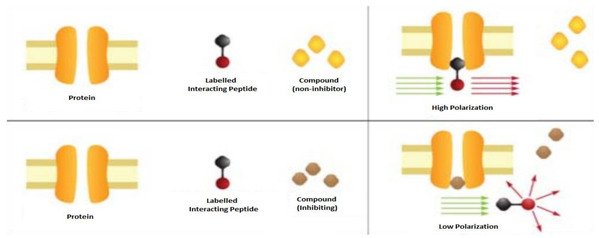
With the increasing number of studies employing microscopy based read outs and quantitative imaging techniques, usage of the facility's imaging platforms has considerably increased. Involvement with clients like radiant research begin at the level of optimizing immunostaining protocols that go all the way upto customizing multiple imaging formats, both on manual as well as automated platforms.

CHEMICAL BIOLOGY UNIT

HIGH THROUGHPUT SCREEN OF SMALL MOLECULE INHIBITORS OF PPI INTERACTIONS

CCBT, Bangalore

Using the Tecan Freedom Evo platform, a high throughput Fluorescence Polarization based screen of inhibitors of cancer-relevant PPIs is being carried out. This screen is carried out against a very large number of small molecules (>100,000 excluding triplicates) from several vendors, and is a demonstration of the scalability of the workflow at the HTS facility.



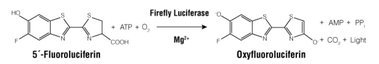
Principle of FP assay, adapted from vendor's webpage

HIGH THROUGHPUT-LUMINESCENCE BASED SCREEN TO IDENTIFY INHIBITORS AND MODULATORS OF THE NOTCH AND HEDGEHOG SIGNALLING PATHWAY

Dr .Syed Sajad Hussain IIIM,Jammu

A luminescence based high throughput screen using 900 compounds(in duplicates) is done to identify those compounds which inhibit the notch signalling pathway and modulate the hedgehog signalling pathway.

The ONE-Glo™ Luciferase Assay System provides a highly sensitive, robust, homogeneous assay for detection of firefly luciferase reporter gene expression in mammalian cells. Ideally suited for high- and ultrahigh-throughput applications, this Assay contains a new luciferase substrate, resulting in a reagent that is more stable, more tolerant to sample components, and has less odor than standard luciferase assay reagents.



Reaction of luciferase assay, adapted from the vendor's webpage

PREVIOUS ACCOMPLISHMENTS

- A High throughput-genome wide-microscopy based RNAi screening strategy was done to identify molecular components of the endocytic pathway in *drosophila* sr+ cells(*Gagan Gupta,Swetha M.G,Gautam Dey,Balaji R.,Sindhu Menon,Revu and others)*
- A High-throughput screen of small molecule inhibitors of interaction of hiv-i encoded nef with cd80/cd86 was performed(*Akanksshi Munjal ,Dr.Satyajit Mayor)*
- Performed a nuclear morphology screen to identify genes that which alter nuclear morphology(*Nisha Ramadas,Dr G.V Shivashankar*)
- Customized imaging protocol for bacterial cells using widefield epi-fluorescence setup(*Astra Zeneca*)

FUTURE DIRECTIONS

- Phase 3 of IISER screen for a set of 2000 genes will continue soon after the data analysis of Phase 2 is done.
- High inflow of genome based RNAi screen is expected to take place.
- Frequent use of the Tecan Infinite M1000 Pro multimode reader for FRET-based and Glow luminescence assays.
- Establishment of basic and advanced training modules on High Content Imaging,High Throughput Screening,Cell culture,Assay design and development is expected to commence by the beginning of 2015.
- An ADME(Absorption, Disposition, Metabolism and Elimination) set-up will soon be functional at the facility.
- Establishment of a repository of a limited number of cell lines.
- Initiating assays and standardization experiments on larger model organisms such as *C.elegans* and zebrafish.

LIBRARIES

DROSOPHILA RNAi LIBRARY 1.0 AND DROSOPHILA RNAi LIBRARY 2.0: Cat No: RDM1189 and RDM4220 **(Open Biosystems)**
The Drosophila RNAi Library-dsRNA templates is a collection of over 15,000 dsDNA templates provided in 96 well PCR plates, ready for in vitro transcription (IVT) to dsRNA constructs that target the entire Drosophila genome. dsDNA templates were generated using gene specific primers to amplify 200-800 bases of exonic sequence for each gene. Individual dsRNAs can be cherry picked from the library and transcribed readily on request.

HUMAN pGIPZ LENTIVIRAL shRNAmir LIBRARY Cat No: RHS5828**(Thermo Scientific/Open Biosystems)**
The Thermo Scientific Open Biosystems GIPZ Lentiviral shRNAmir Library was developed in collaboration with Dr. Greg Hannon (CSHL) and Dr. Steve Elledge (Harvard). This library combines the design advantages of microRNA-adapted shRNA (shRNAmir) with the pGIPZ lentiviral vector to create a powerful RNAi trigger capable of producing RNAi in most cell types including primary and non-dividing cells.
The individual clones of shRNA are readily available as glycerol stocks.

Faculty Advisors for the Screening Facility – Dr Apurva Sarin, Dr Satyajit Mayor, Dr. Varadharajan Sundaramurthy

ACKNOWLEDGEMENTS

IISER screen: Lokavya M Kurup,Vandana .G.Pawar, Dhruv Raina, Balaji Ramalingam, Lokesh Pimpale
PPI Screen – SubbuRao Jasti,Chandan Atreya,Manjunath.H,Dhruv Raina,Lokavya M Kurup,Chandni R
DDR Screen– Suranjana M,Dhruv Raina, Lokavya M Kurup, Shruthi N, Pragya J

REFERENCES

Detailed information on various instruments of the Screening Facility available at the respective company websites :–
Thermo Scientific Cellomics ArrayScan VTI - http://www.cellomics.com/
Tecan - http://www.tecan.com/
Invitrogen - http://www.lifetechnologies.com
Bio-Rad-http://www.bio-rad.com
Nikon TiE- http://www.nikoninstruments.com

FUNDING

We wish to thank The Nanoscience Mission (Department of Science and Technology), and the HTS Program (Department of Biotechnology) for generously funding RNAi reagents for the Screening facility.

Current members of the Screening Facility

Technology Manager-Dr. Shahabuddin.M.S
Technology Associate-Chandan Mithra
Senior Technology Associate-Lokavya M Kurup
Technology Assistant-Vandana.G.Pawar

Previous Members of the Screening Facility

Dhruv Raina, Balaji Ramalingam, Giridharan Periyasamy, Joseph Mathew, Gagan.D.Gupta,Priya and Nisha Ramadas