

RNAi Screening Facility

RNAi Screening/Functional Genomics/High Content Imaging Facility

The IMCB RNAi facility encompass RNAi screening using siRNA arrayed library provided by us, related equipments such as contact and non contact dispensing robots, automated high content imagers, TC room, expert advice and help. We are localized in Proteos #5-12 #5-13

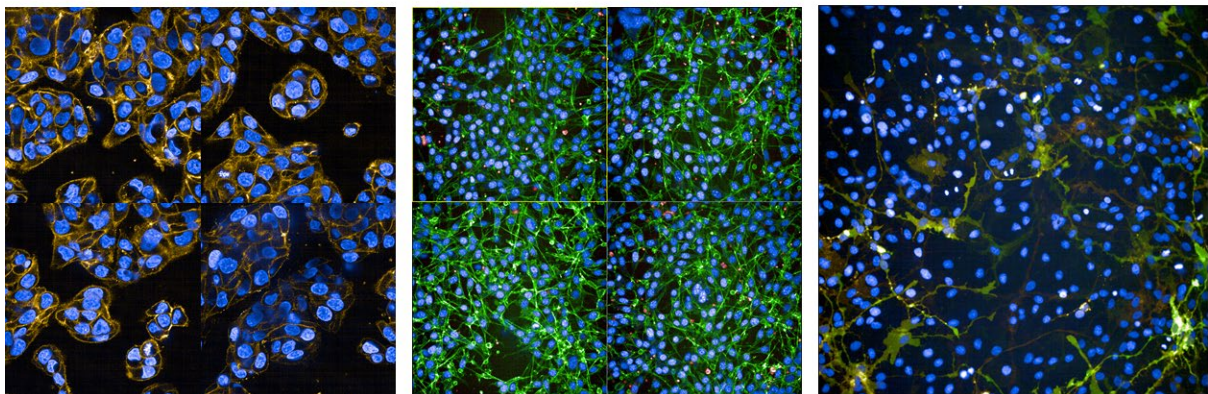
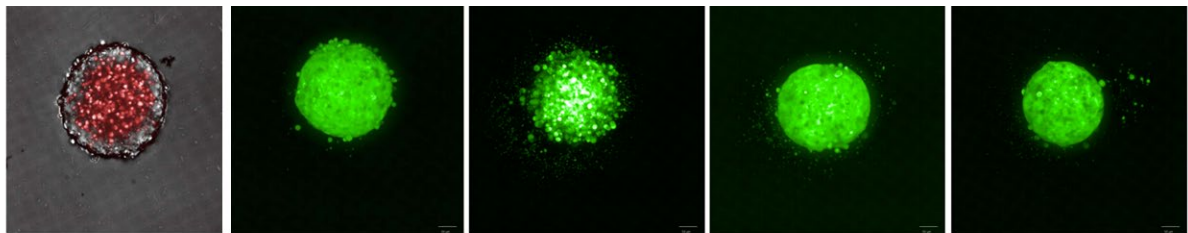
The IMCB RNAi screening facility was established within [Frederic Bard's laboratory](#) in 2005 and was the first center in Singapore performing genome wide RNAi screening.

Philosophy of IMCB RNAi screening center

- * **Open to researchers in Singapore**
- * **Do-it-yourself facility**
- * **Facility provides siRNA libraries in assay 384 well plates, screening equipment, Tissue Culture room and support.**

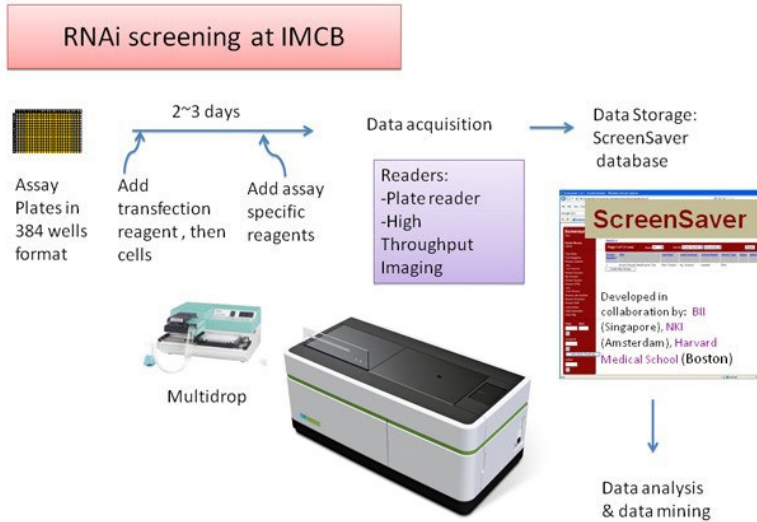
The overview of screening infrastructure includes:

1. [Screening facility workflow and equipments](#)
2. [List of siRNA Library](#)
3. [RNAi screen experiment support](#)
4. [Bioinformatic support](#)
5. [Contact persons](#)



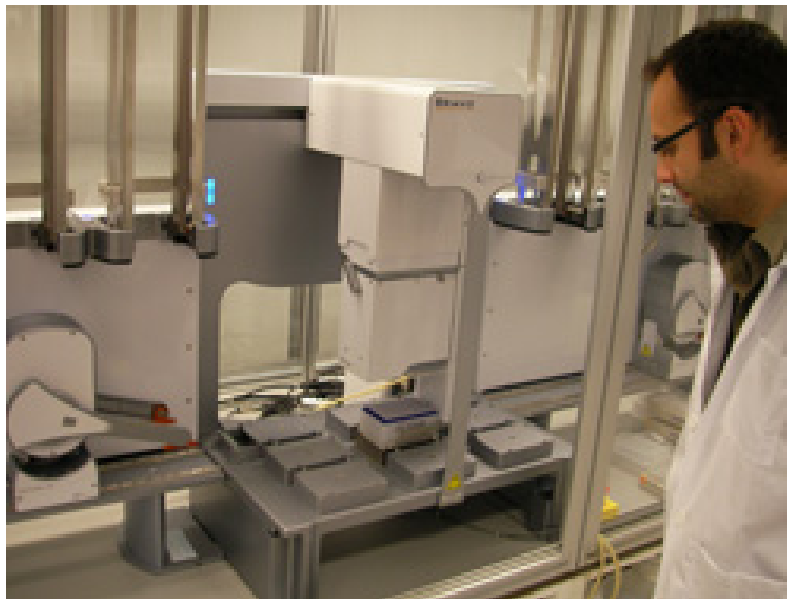
Screening facility workflow and equipments

Overview of screening workflow



Velocity Plates arraying :

The arrayed siRNA assay 384 well plates are prepared with velocity plate robot(Agilent Bravo V11). Each well contains a SMARTpool (4 siRNA) against a particular gene.



Assay preparation: The screener prepares his own reporter cell lines and transfection mix with his own reagents.



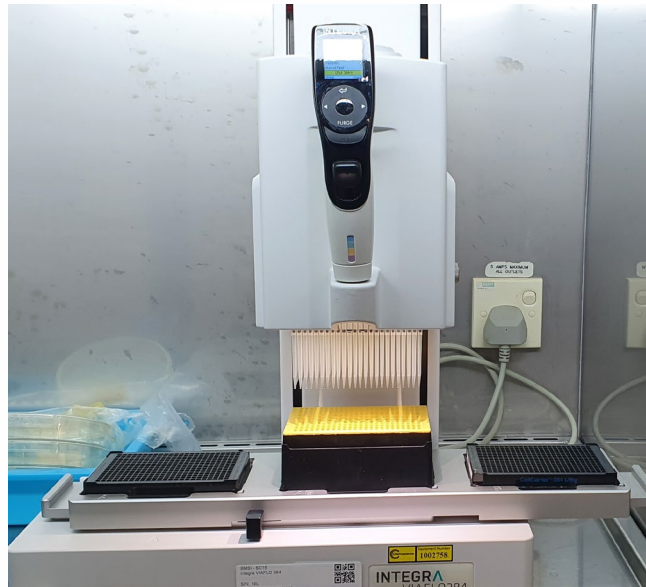
Multidrop combi :

The screener can distribute his cells into the siRNA assay 384 well plates with the multidrop combi who is a peristaltic pump non contact dispenser. Users can also distribute staining reagent or transfection reagents throughout plate with such systems. We currently have 3 multidrop combi systems.



384 Viaflo (Integra Biosciences)

384 Viaflo is a contact dispenser who can serve more customized workflow such as forward transfection, transfer of cells from 384 to 384 plate or recovery of media supernatant from functional genomics screen. System is placed in a tissue culture hood. Tips for such customized workflow are chargeable to users.



Perkin Elmer High content imaging suites with automated plate loading with robotic arm :

We are equipped with an Opera Spinning Disk confocal Phenix system (Perkin Elmer), laser based, 4 colors, 2 cameras, 5x air, 20X air, 20X water immersion, 40X water immersion, 63X water immersion. System is compatible with imaging grade plates (less than 1mm thickness, less than 22mm total height) for 6, 12, 24, 96 and 384 well plates and slides. Preciscan option available. System used fixed sample only.

<https://www.perkinelmer.com/product/opera-phenix-plus-system-hh14001000>

We are also equipped with an Operetta CLS system (Perkin Elmer), LED based, 4 colors, 1 camera, 5X air, 10X air, 20X air, 20X water immersion with environment chamber. System is compatible with imaging grade plates (less than 1mm thickness, less than 22mm total height) for 6, 12, 24, 96 and 384 well plates and slides. System used fixed and live samples.

<https://www.perkinelmer.com/category/operetta-cls-high-content-analysis-system>

Both systems are connected to an automated robotic arm who can do barcode reading and load system with up to 14 plates.



Imaging Data is pushed to Columbus server Imaging storage and analysis system who can perform analysis from any computer connected to IMCB intranet network.

<https://www.perkinelmer.com/product/image-data-storage-and-analysis-system-columbus>

Well	Row	Column	Field	Image	Timepoint	Plane	Channel	Channel Name	Channel Color	Channel Type	Acquisition Type	Image Resolution X [µm]	Image Resolution Y [µm]	Image Resolution Z [µm]	Main Emission Wavelength [nm]	Main Excitation Wavelength [nm]
C3	3	3	1	5081860	1	0	2	Alexa 488	0x3e490	Fluorescence	SpinningDiskConfocal	0.298988040478	0.298988040478	1	522	48
C3	3	3	1	5081860	1	0	3	Alexa 647	0x054545	Fluorescence	SpinningDiskConfocal	0.298988040478	0.298988040478	1	706	64
C3	3	3	1	5081860	1	0	1	Hoechst 33342	0x006666	Fluorescence	SpinningDiskConfocal	0.298988040478	0.298988040478	1	456	42

High content imaging system: IncuCyte® ZOOM Live-Cell Analysis System

We have also an Incucyte zoom available with 10X objective, brightfield, green and red fluorescence capability. System can acquire live imaging experiments for a week with up to 6 multiwell plates acquisition in parallel. Scratch would healing module available

<https://www.essenbio.com/en/resources/incucyte-zoom-resources-support/>



Plate Reader: Tecan Infinite M200

The screener with luminescence and fluorescent assays can use plate reader to generate data readout of his results.



* Other equipments available for use

- >> Dedicated tissue culture room
- >> access to vicell counter for establishing cell counts
- >> 1 Large TC hood
- >> TC incubators
- >> Extra freezers and fridges

List of siRNA Library in siGenome SMARTpool arrayed format (Horizon discovery)

- >> Human kinome/phosphatome
- >> Mouse kinome
- >> Human genome
- >> Mouse full genome

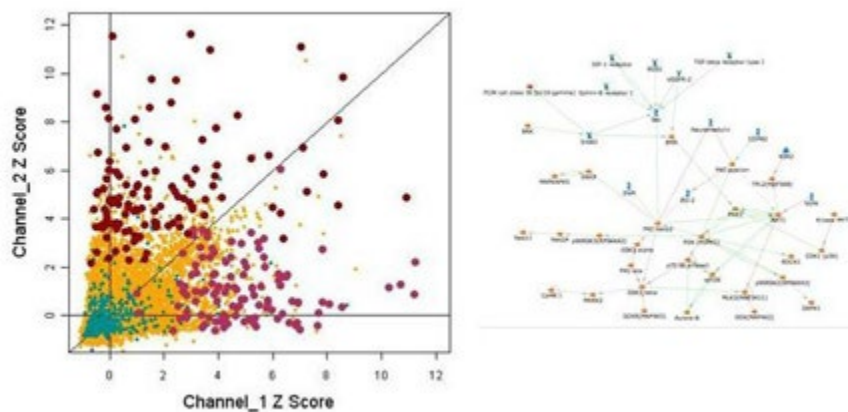
RNAi screen experiment support

The screener can get expert help from FB lab personnel experienced in functional genomic screen: Hui Hui, Joanne, Jeremy, Felicia and Xavier. They can help the screener at various stage like designing plate for experiment, assay preparation and data acquisition.

Bioinformatic analysis support

We will perform analysis of the data and normalisation. We have developed ScreenSifter as a solution to analyse RNAi data.

<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-14-290>



Please contact

RNAi Facility Manager: LE GUEZENNEC Xavier (lgxavier@imcb.a-star.edu.sg) .