Introduction

With complete sequence of yeast genome in 1996 and construction of single-deletion collection in year 2000, era of whole-genome experimental approaches started to evolve with high speed. One of early techniques that emerged was Synthetic Genetic Array (SGA). Crossing of query strain, which carries a mutation in gene of interest, with desired collection, sporulation and selections for specific markers, enable us to introduce new perturbation in vast number of strains relatively fast. Newly introduced mutation represents a hub in genetic network, whose interactors we would like to annotate. In addition, external perturbations, e.g. drugs or non-permissive temperature, can also give additional information about system’s response. With usage of many different libraries we can study effects of depletion, over-expression and localization of gene products in cells under specific conditions. Today SGA represents a very powerful tool in the field of yeast functional studies. We established it as a platform at Institut Curie, available to all interested research groups.

Aims

- Identifying genetic interactions between genes involved in individual pathways
- Studying gene or protein functions
- Elucidating mechanism of action of drugs

Services

Agar-based assays:

- chemical genomics
- synthetic genetic array
- E-MAP
- ...

Liquid-based assays:

- growth curves
- colorimetric assays
Equipment

Robotic manipulator BM3-BC (S&P Robotics, Inc.) with a 48 plate capacity is used for transferring cells from/to solid and liquid media in 96, 384 and 1536-well formats. It includes a station for fully automated image acquisition. Additionally, it was upgraded with a re-arraying tool that enables custom-designed organization of collections or isolation of individual strains from plates. It represents a core tool for high-throughput mutant construction and chemogenomic testing.

Workstation for liquid-based experiments is comprised of an Eon microplate reader and a BioStack automated plate handler with a 30 plate capacity (BioTek), placed in a custom-built incubator (Jim Engineering). The spectrometer, which has a thermostatic chamber, reads 6- to 384-well microplate formats, within the 200 to 999 nm wavelength range. The workstation can be used for follow-up experiments after agar-based experiments, or as a stand-alone method for monitoring growth with high resolution under different conditions. Wide continuous wavelength range makes it a perfect tool for colorimetric tests, for which many kits are available on the market.
Additional equipment the platform offers which is indispensable for experimental work: -80°C freezer, centrifuge, two incubators, electronic multi-channel pipettes (96 and 12-channel for different volume range), microscope, iTrack pipette tracking system and a plate shaker.

Yeast collections

*S. cerevisiae* collections that the platform maintains and provides to users:

- single deletion collection (deletions of non-essential genes),
- temperature sensitive mutants (mutations of essential genes),
- decreased abundance by mRNA perturbation (mutations of essential genes),
- H3/H4 single histone point-mutations,
- VN-fusion library (protein-protein interactions based on bimolecular fluorescence
Data analysis

Data analysis is performed at the platform upon request. It comes in different flavors: from raw data processing and quality control to biological insights. Even though many steps of the analysis are automated, project-specific steps are implemented regularly. To increase data sharing and knowledge transfer, all software used is open access (R, Cytoscape, web apps for gene ontology enrichment etc.) and developed scripts are available to users.

Key publications

Year of publication 2018

Antoine Hocher, Myriam Ruault, Petra Kaferle, Marc Descrimes, Mickaël Garnier, Antonin Morillon, Angela Taddei (2018 Oct 26)

Expanding heterochromatin reveals discrete subtelomeric domains delimited by chromatin landscape transitions.

Genome research: DOI: gr.236554.118