Simplified workflow for phenotypic profiling based on the Cell Painting assay

Introduction

Multiparametric high-content screening approaches, such as the Cell Painting assay, are increasingly being used in many applications ranging from drug discovery programs to functional genomics screening. The Cell Painting assay uses up to six fluorescent dyes to label and visualize a variety of organelles at the single-cell level. All the features extracted from the assay give unique cellular “signatures” that characterize any given cell. In addition, insights into the mechanism of action may be gained by comparing the phenotypic profiles of novel compounds with those reference compounds. Most biologists are familiar with the methods used in the Cell Painting assay protocol. However, the sheer volume of data generated from these experiments require additional computational tools to extract meaningful information.

Here, we present a complete workflow for the Cell Painting assay that can be easily implemented. Using this approach, we find that cells treated with the same compound show similar phenotypic profiles. Hierarchical clustering analysis grouped highly toxic compounds such as paclitaxel and etoposide, latrunculin B, rapamycin, rotenone, staurosporine, paclitaxel and tetrandrine. Each of these compounds are clustered together (Figure 4). Hits from these clusters were selected for further analysis.

Methods

Overview of the workflow for image-based profiling. Details for each step listed below.

1. U2OS cells (ATCC) were seeded at 2000 cells per well.
2. Eleven compounds were tested in quadruplicate wells in a seven point 1:3 dilution series along with suitable controls. Compounds used: CA-074-Me, CCCP, chloroquine, cytochalasin D, etoposide, latexrinin B, rapamycin, rotenone, staurosporine, paclitaxel and tetrandrine.
3. Twenty four hours after compound treatment, cells were stained with the cell painting dyes using the protocol by Bray et al. The following dyes were used: MitoTracker Deep Red, wheat germ agglutinin/Alexa Fluor 555, Concanavalin A/AlexaFluor 488, phalloidin/Alexa Fluor 568, SYTO14 and Hoechst 33342.
4. Image acquisition was performed on the ImageXpress® Micro Confocal High-Content Imaging System (Molecular Devices) using a 20X Plan Apo objective, with the following filters ex/em: DAPI 377/447, FITC 475/536, TRITC 543/593, TexasRed 560/624, Cy5 631/692.
5. IN Carta® Image Analysis Software was used for image analysis. 280 measurements that were selected include parameters related to intensity, texture, shape, spatial relationship, and co-localization scores.

Data analysis workflow

Measurements from the IN Carta software were uploaded into HC StratomeR® for further data analysis. Briefly, quality control, plate normalization, data transformation, and feature standardization was applied. Principal component analysis (PCA) was used to reduce the dimensionality of the data set. Further downstream analyses such as hit selection and cluster analysis were performed based on the principal components and the phenotypic distance score derived.

Results

Scatter plot shows interactions between data points in relation to three different PCAs.

Comparison of phenotypic profiles – Clustering

The distance score is calculated based on selected principal component scores. This score represents the phenotypic distance a sample is from the negative control which can be used for hit selection in screening assays. Subsequently, hierarchical cluster analysis can be performed based on selected features or compounds.

Conclusion

- Our results demonstrate the feasibility of using ImageXpress Micro Confocal system, IN Carta software, and StratomeR® for image-based profiling assays.
- The IN Carta software combines ease of use with more advanced feature segmentation options (SINAP) to allow robust segmentation of cellular features.
- The StratomeR® platform allows non-expert users to rapidly carry out phenotypic data analysis with its intuitive guided workflow.

References

1. Bray MA et al. Nat Protoc. 2016 Sep;11(9):1755-74
2. Going K et al. /Bio Chem 2014 Oct 22;43842:2615-2616