Cell Painting for compounds clustering and Mechanism Of Actions characterization

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Graphical abstract

Cell Painting methodology overview. Automated image analysis generates compound profiles. After comparison of all profiles, t-SNE visualization is used to evidence clustering of compounds with similar effect.

Introduction

Cell Painting is a multi-parameter image-based description of the cell response to any perturbator condition: treatment with a compounds, a siRNA, CRISPR engineering, ....

We are now using Cell Painting to support hit triage at the end of a High Throughput Screening, in order to select series with optimized phenotypic characteristics, for example to avoid major off-target effect or to cluster compounds with similar impact on the cells.

Conclusions and next steps

We have established a robust workflow to characterize with Cell Painting several thousands of compounds. Automated processes have been developed for the wet biology part as well as for data processing. After extensive quality control, data can be manipulated and visualized under different views in Spotfire.

We first confirmed the power of the technology to evidence groups of compounds with similar mechanisms of action. Among 5,200 bioactive compounds, we selected a set of 225 molecules representing 57 major phenotypic groups. Interestingly, when tested against different cell lines and at different time points, this set has shown reproducible clustering of compounds targeting similar pathways.

We are now using Cell Painting to support hit triage at the end of a High Throughput Screening, in order to select series with optimized phenotypic characteristics, for example to avoid major off-target effect or to keep some degree of biological diversity.

Applications of Cell Painting

- Predict the MOA of unannotated compounds by comparison with a set of reference compounds
- Group a large collection of unannotated compounds into clusters that harbor the same MOA
- Help maximize compounds profile diversity during hit triaging
- Identify compounds with potentially new MOA
- Identify compounds that revert to a control condition from a treated condition
- Identify cell line specific effect by comparing the compounds’ profiles across different cell lines
- Follow evolution of similarity to reference compounds upon compound concentration increase
- Identify concentrations of compounds that have off-target effects

Results

- 850 compounds are detected as active out of 5,200 compounds
- Clear clustering of compounds sharing similar targets/pathways
- Comparison with hierarchical clustering is needed to correct some artifacts of the t-SNE projection
- Duplicate of the experiment shows very robust phenotypes
- Same collection tested on other cell line still grouped by major function: robust selection
- 225 compounds, representing 57 major groups were selected as reference set

Quality Control

1. Sufficient cell count
2. Low variability for control wells
3. Control conditions mostly inactive
4. No time wise shift
5. No pattern due to cell seeding
6. No outliers values in profiles
7. No correlation between plate compound localization and t-SNE output
8. Even contribution of 4 channels to selected features
9. No spread of replicates in the t-SNE (non aggregated values)
10. Grouping of similar MOAs (aggregated values)

Methodology

Wet biology

- Compound treatment
- 24 hours cell treatment
- 4.or + doses
- Reference cpds

Image acquisition/analysis

- Neutrons extraction
- Autofluorescence
- Phalloidin
- Mitotracker
- ... markers: Hoechst, Phalloidin, Mitotracker, ConcanavalinA

Image analysis/visualization

- Python, R, C, Spotfire
- Stages: segmentation, texture, cumulative, intensity, nucleus, cytoplasm, ...
- Text screens: t-SNE colored by plate
- T-SNE on 5204 compounds tested at 4 concentrations

Applications of Cell Painting

- Use artificial intelligence for image analysis
- Replace well level by cell level analysis
- Select features
- Normalize
- Visualize
- Tidy

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