# Examining the Effects of Reference Chemicals Across A Variety of Human-Derived Cell Lines Using High-Throughput Phenotypic Profiling (HTPP)

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## Background

- HTPP is a chemical screening method that combines high-content imaging and image analysis to measure phenotypic features at the single cell level and quantify chemical effects on morphology.
- Used as an efficient and cost-effective method for evaluating the bioactivity of environmental chemicals with no requirement for *a priori* knowledge of molecular targets.
- This project aimed at identifying 20 candidate phenotypic reference chemicals (RefChem20) as plate-based controls for future screening studies.
- These compounds were tested in 12 human-derived cell lines identified using a data-driven cell line selection approach:

| Cell Line | Tissue Origin | Disease State | Morphology |
|---|---|---|---|
| ARPE-19 | Retinal; pigmented | Normal (Primary) | epithelial |
| ASC52Telo | Adipose | Normal (hTERT Immortalized) | fibroblast-like |
| BJ-5ta | Skin; foreskin | Normal (hTERT Immortalized) | fibroblast-like |
| CCD-18Co | Colon | Normal (hTERT Immortalized) | fibroblast-like |
| CHON-001 | Long bone; cartilage | Normal (hTERT Immortalized) | fibroblast-like |
| HBEC3-KT | Lung; bronchus | Normal (hTERT Immortalized) | epithelial |
| HME-1 | Breast; mammary gland | Normal (hTERT Immortalized) | epithelial |
| Ker-CT | Skin; foreskin | Normal (hTERT Immortalized) | epithelial |
| RPTEC | Kidney; proximal tube | Normal (hTERT Immortalized) | epithelial |
| TeloHAEC | Heart; aorta | Normal (hTERT Immortalized) | endothelial |
| MCF-7 | Breast | Adenocarcinoma | epithelial |
| U-2 OS | Bone | Osteosarcoma | epithelial |

## Experimental Design

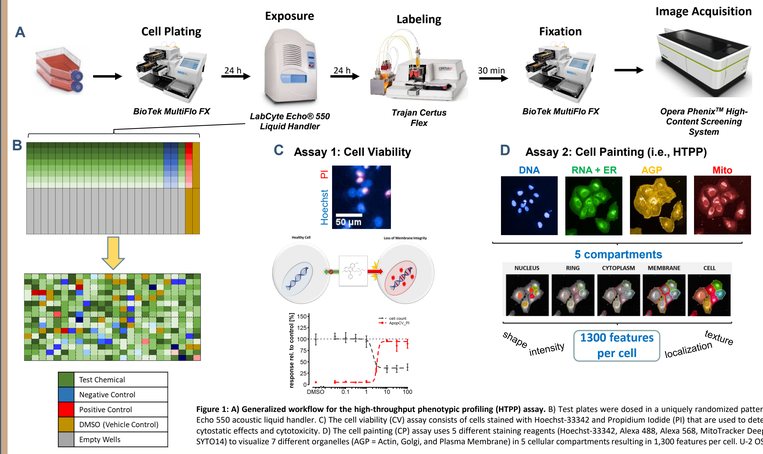



**Figure 1: A) Generalized workflow for the high-throughput phenotypic profiling (HTPP) assay.** B) Test plates were dosed in a uniquely randomized pattern using an Echo 550 acoustic liquid handler. C) The cell viability (CV) assay consists of cells stained with Hoechst-33342 and Propidium Iodide (PI) that are used to determine cell cytostatic effects and cytotoxicity. D) The cell painting (CP) assay uses 5 different staining reagents (Hoechst-33342, Alexa 488, Alexa 568, MitoTracker Deep Red, and SYTO14) to visualize 7 different organelles (AGP = Actin, Golgi, and Plasma Membrane) in 5 cellular compartments resulting in 1,300 features per cell. U-2 OS shown.

## Results: Comparison of Chemical Heatmaps

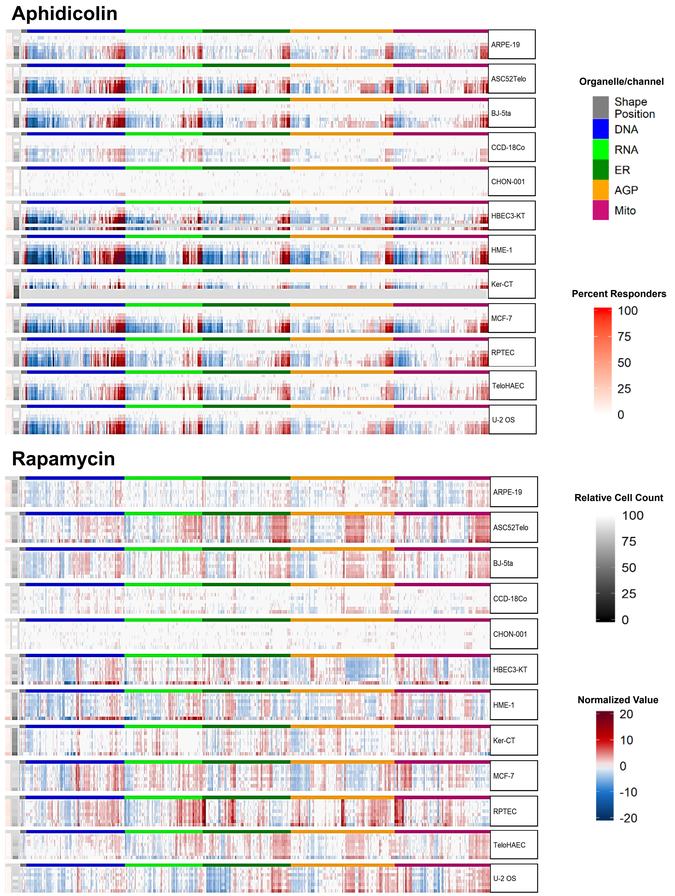
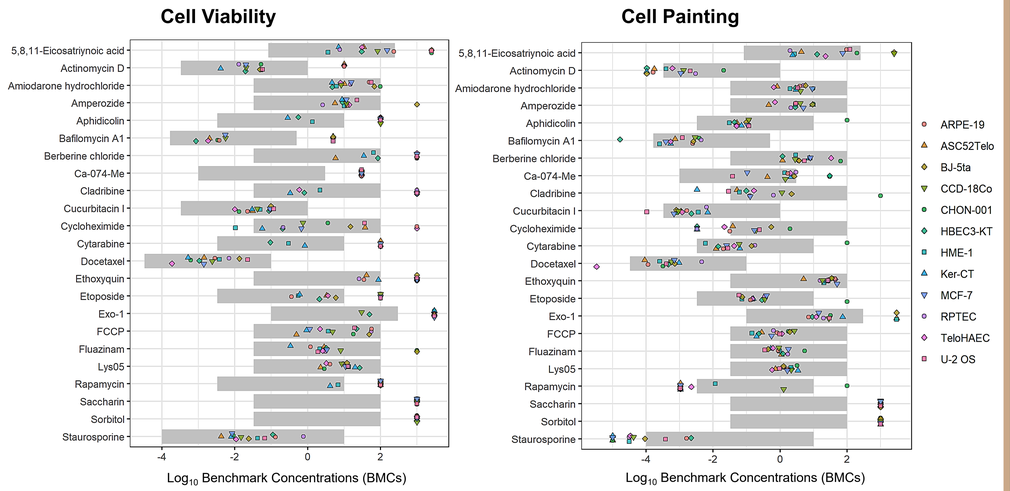



**Figure 2: Examples of two chemical heatmaps with results displayed across all 12 cell lines tested.** The heatmap on the top shows results from aphidicolin where features present in all organelle channels appear to be strongly affected by chemical treatment in a consistent pattern across cell types. The heatmap on the bottom shows results from rapamycin where feature effect sizes are smaller in comparison to aphidicolin and differences in magnitude and directionality of effect can be seen in channels for some cell types (e.g. the AGP channel in the ASC52Telo and BJ-5ta cell lines display a strong red signal while the signal tends to be a light blue in the HBEC3-KT and MCF-7 cell lines).

## Results: Cell Line Comparison of Chemical Benchmark Concentrations (BMCs)




⇨ **Cell Painting BMCs < Cell Viability BMCs**

⇨ **BMCs were found to be within the tested dose range for majority of reference chemicals**

**Figure 3: Comparison of benchmark concentrations (BMCs) across all cell lines tested with both cell viability (CV) and cell painting (CP) results. The tested dose range for a particular chemical is highlighted by the gray box.** The 20 reference chemicals were tested in a total of 12 different cell types with varying tissues of origin. In additional to the reference chemicals, there were also two negative controls tested (i.e. saccharin, sorbitol), as well as a cell viability positive control chemical (i.e. staurosporine). For some chemicals, such as amperozide and Lys05, the BMCs across cell types appear to be consistent while other chemicals, such as cladribine and cycloheximide, the BMCs are separated by several orders of magnitude. The BMCs for the cell painting data tend to be left-shifted when compared to the BMCs for the cell viability data. BMCs determined from both assay types tend to fall inside the tested dose range for majority of chemicals.

## Conclusions and Future Directions

⇨ **The morphological phenotypes of the reference chemicals were consistent with those reported in the scientific literature for the Cell Painting assay.**

⇨ **For many chemicals, comparable potencies and phenotypic profiles were observed across all cell types tested using the Cell Painting assay.**

⇨ **One particular cell line (e.g. CHON-001) showed a decreased sensitivity to chemicals known to affect the cell cycle.**

⇨ **These data will facilitate selection of appropriate phenotypic reference chemicals for use in larger screening studies involving these cell lines.**

⇨ **Future directions include screening these cell types using expanded chemical sets and incorporating the resulting information into existing approaches for hazard binning and prioritization using *in vitro* screening data.**

⇨ **Currently screening 1470 chemical in the HBEC3-KT and TeloHAEC cell lines using the HTPP assay with results expected in 2022.**