Optimization of culture conditions and identification of reference chemicals for combination screening using TempO-Seq and Cell Painting

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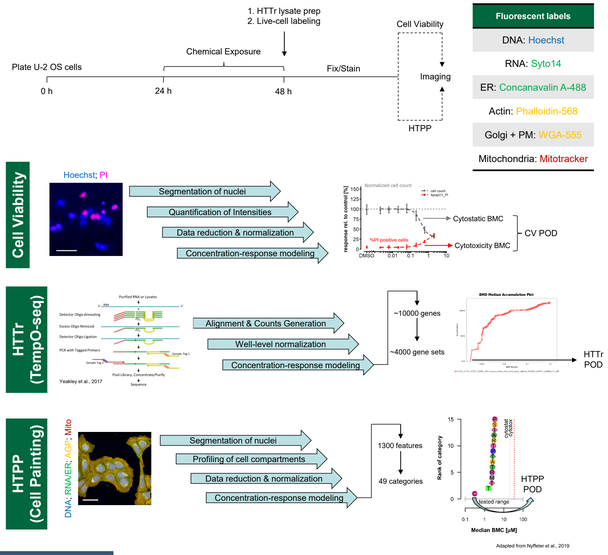
Abstract

The recently released Next Generation Blueprint for Computational Toxicology at the USEPA advocates the use of broad-based high-content profiling assays as a first step for characterizing the biological activity of environmental chemicals. Two such high-throughput profiling approaches being evaluated are whole transcriptome targeted RNA-Seq (i.e. TempO-Seq) and high content imaging-based phenotypic profiling (i.e. Cell Painting), both of which can be applied to a variety of human-derived cell types. This work describes the optimization of the U-2 OS human osteosarcoma cell model for combination screening with TempO-Seq and Cell Painting. First, a time course experiment was performed in 384-well format to identify an initial seeding density (i.e. 3,000 cells/well) that would yield enough cells / well to satisfy TempO-Seq lysate requirements (i.e. $0.25x10^6$ – $2x10^6$ cells / mL lysate) and not result in overly-confluent monolayers for Cell Painting at 48 h, post-seeding. Next, a set of eleven chemicals with known molecular modes-of-action were screened in concentration-response mode (n = 7 concentrations, ½ log10 spacing) in order to identify a set of three phenotypic / gene expression reference chemicals for use in evaluating TempO-Seq and Cell Painting assay performance during large-scale screening campaigns. Following 24 h of treatment, each candidate reference chemical produced concentration-dependent changes in phenotypic profiles in the Cell Painting assay that were similar to those previously observed in experiments using lower initial seeding densities (400 cells / well). The benchmark concentration for onset of phenotypic changes was similar between low and high density cultures whereas benchmark concentrations for cytotoxicity were right-shifted at the higher cell density. Baseline gene expression profiling of U-2 OS cells with whole transcriptome TempO-Seq confirmed expression of the glucocorticoid (NR3C1) and retinoic acid (RARA) receptors. Well-characterized agonists of these receptors (dexamethasone and all-trans-retinoic acid , respectively) produced concentration-dependent changes in the mitochondrial and endoplasmic reticulum morphology. These chemicals, plus the topoisomerase II inhibitor etoposide, were selected as reference chemicals for high-throughput screening studies in the U-2 OS cell model.

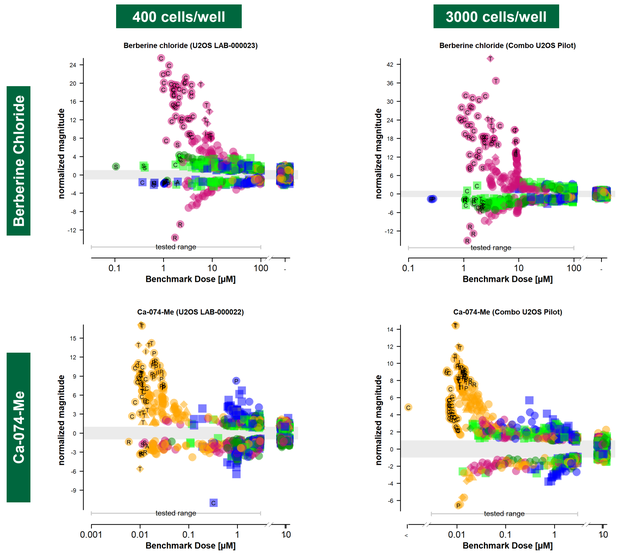
Experimental Design



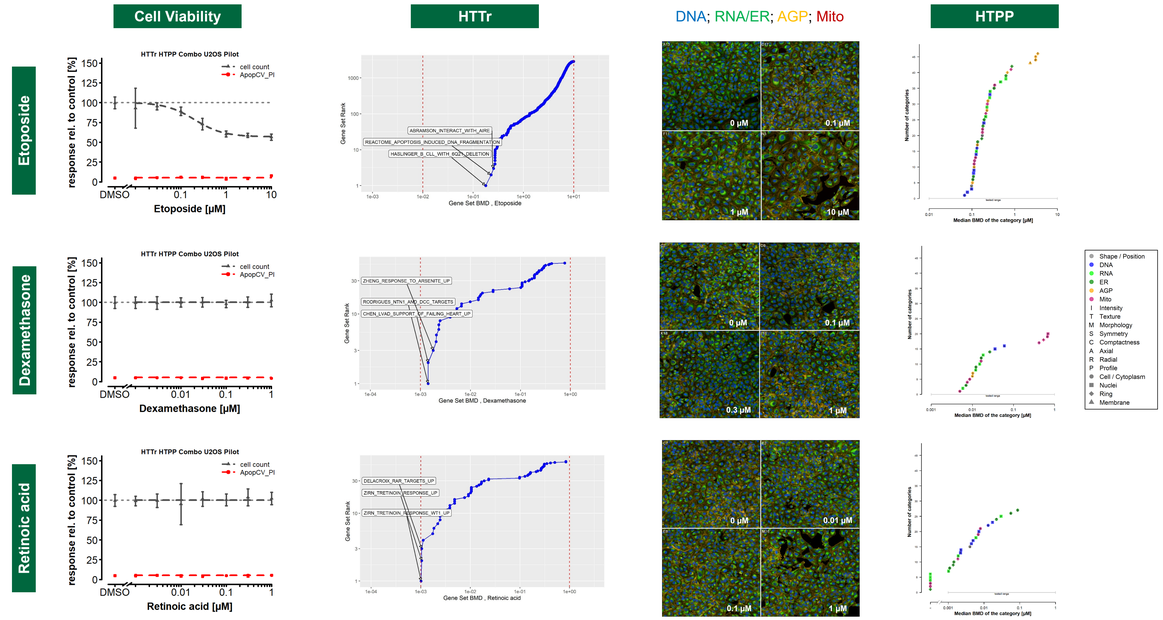
Reference Chemicals

	DTXSID	Chemical Name	Rationale	Mechanism/Target	Concentration Range (µM)
1	DTXSID3020384	Dexamethasone	Evidence for effects on gene expression in osteosarcoma cells	Glucocorticoid receptor (NR3C1)	0.001-1
2	DTXSID6024882	Cycloheximide	Large HTTr effects (in MCF7 cells)	Protein translocation	0.01-10
3	DTXSID9020031	Actinomycin D	Large magnitudes (RNA channel)	RNA polymerase	0.0001-0.1
4	DTXSID5023035	Etoposide	HTPP reference chemical	DNA topoisomerases	0.01-10
5	DTXSID7021239	Retinoic acid	Evidence for effects on gene expression in osteosarcoma cells	Retinoic acid pathway	0.001-1
6	DTXSID6037063	Trichostatin A	HTTr reference chemical	HDACs	0.003-3
7	DTXSID0040464	Docetaxel	Large magnitudes (DNA channel)	Microtubule stabilization	0.00003-0.03
8	DTXSID501015546	Cucurbitacin I	Large magnitudes (AGP channel)	STAT3/JAK	0.0001-0.1
9	DTXSID8024602	Berberine chloride	HTPP reference chemical	Mitochondrial toxicant	0.1-100
10	DTXSID50881386	Ca-074-Me	HTPP reference chemical	Cathepsin B	0.003-3
11	DTXSID5023582	Rapamycin (Sirolimus)	HTPP reference chemical	mTOR	0.001-1

Higher cell density yields similar phenotypic profiles



Chemicals selected as in-plate reference chemicals for High-Throughput Screening in U-2 OS cells



Points of Departure (PODs)

Chemical Name	Cell Viability (uM)	HTTr (uM)	HTPP (uM)	POD Ratio (HTPP / HTTr)
Dexamethasone	n/a	1.42E-03	5.01E-03	0.3
Cycloheximide	n/a	1.40E-01	1.04E-01	1.3
Actinomycin D	2.34E-02	9.62E-04	5.37E-04	1.8
Etoposide	n/a	1.78E-01	6.76E-02	2.6
Retinoic Acid	n/a	1.01E-03	3.16E-04	3.2
Trichostatin A	2.26E-01	1.02E-01	2.32E-02	4.4
Docetaxel	7.08E-03	2.04E-03	1.66E-04	12.3
Cucurbitacin I	6.61E-02	1.22E-02	6.92E-04	17.6
Berberine chloride	n/a	4.45E+01	1.81E+00	24.5
Ca-074-Me	n/a	3.30E-01	7.85E-03	42.0
Rapamycin (Sirolimus)	n/a	8.18E-02	3.16E-04	258.8

Conclusions

- Cucurbitacin I cell viability concentration-response curve was right-shifted at the higher cell seeding density, possibly indicating reduced sensitivity; all other cell viability concentration-response curves were similar at the lower and higher cell seeding densities
- HTPP concentration-response profiles were similar at the lower and higher cell seeding densities
- Etoposide, dexamethasone, and retinoic acid were selected as in-plate reference chemicals for high-throughput screening in U-2 OS cells
- None of the reference chemicals had a cell viability POD in the concentration range tested; however, etoposide reduced cell number
- The most sensitive gene sets for each reference chemical were consistent with their known biological activity
  - Etoposide: REACTOME_APOPTOSIS_INDUCED_DNA_FRAGMENTATION
  - Dexamethasone: BURTON_ADIPOGENESIS_1
  - Retinoic acid: DELACROIX_RAR_TARGETS_UP
- The phenotypic profile of each reference chemical was distinct
- HTPP was more-sensitive at detecting the biological activity of the chemicals as compared to HTTr, in most cases

Future Directions

- Concentration-response screening of ToxCast chemicals in U-2 OS cells using both HTTr and HTPP
- Exploring potential alternative methods for concentration-response modeling and POD determination