Concentration-Response Screening of Environmental Chemicals for In Vitro Point-of-Departure Determination using a Targeted RNA-Seq Whole Transcriptome Assay

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1 Background and Objectives

Background

- Current initiatives in environmental toxicology include integrating toxicity testing data from *in vitro* and alternative methods into risk assessment practices.
- Previously, *in vitro* point-of-departure (PODs) estimates suitable for use in screening-level risk assessments have been based on panels of *in vitro* assays that report a limited number of endpoints and that may not provide comprehensive coverage of all biological pathways.
- Increasing efficiency and declining cost of generating whole transcriptome profiles has made high-throughput transcriptomics (HTTr) a practical option for determining *in vitro* bioactivity thresholds and increasing coverage of biological space in chemical screening.

Objectives

- Develop a laboratory workflow for concentration-response screening of environmental chemicals using a targeted RNA-Seq human whole transcriptome assay (**TempO-Seq**).
- Perform concentration-response screening of a large number of chemicals (n=2,200) from the USEPA Toxicity Forecaster (ToxCast) compound library in MCF7 cells at a single time point (6 hours post-exposure).
- Evaluate the performance of the targeted RNA-Seq assay across plates and screening blocks using reference samples (i.e. commercially-available purified RNAs & laboratory generated bulk cell lysates) and treatment with reference chemicals.
- Establish a data processing pipeline for data normalization, differentially expressed gene (DEG) identification and determination of *in vitro* PODs.

2 Laboratory Workflow

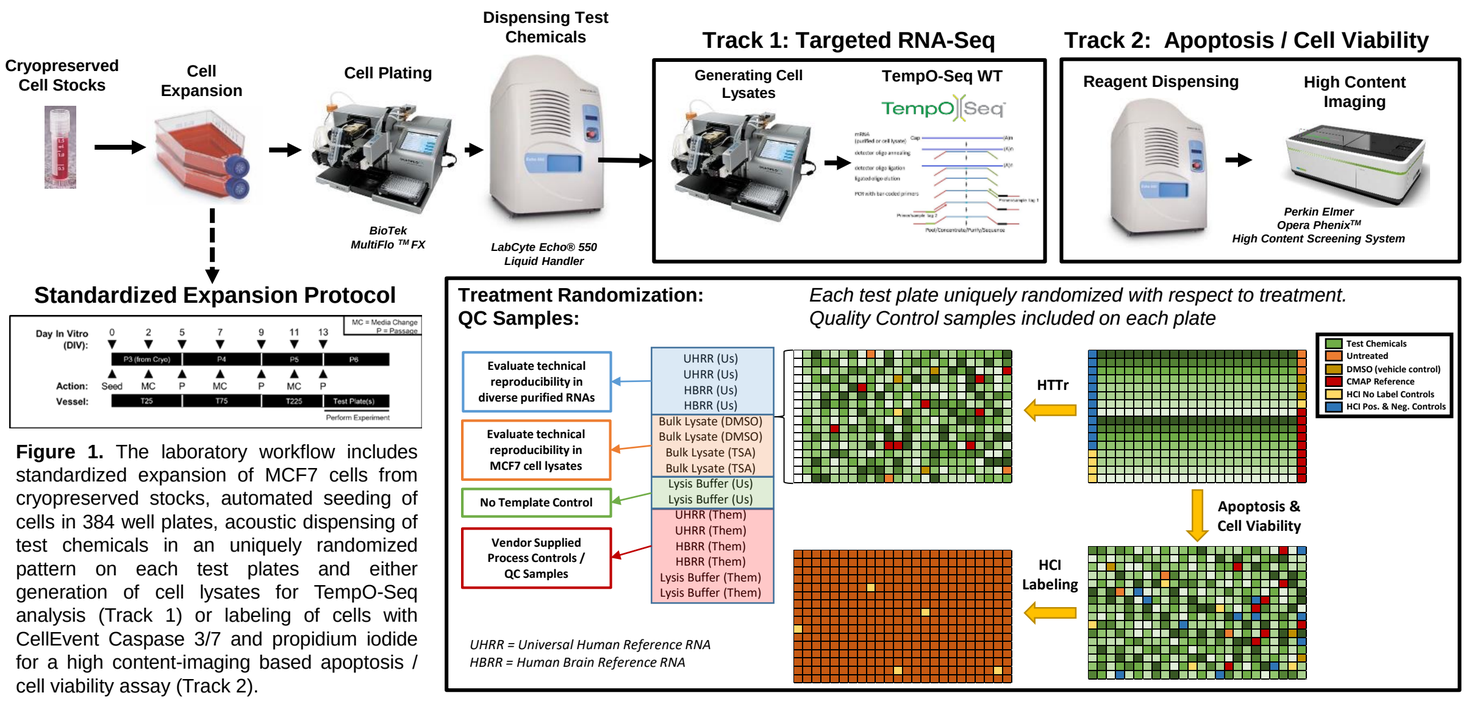
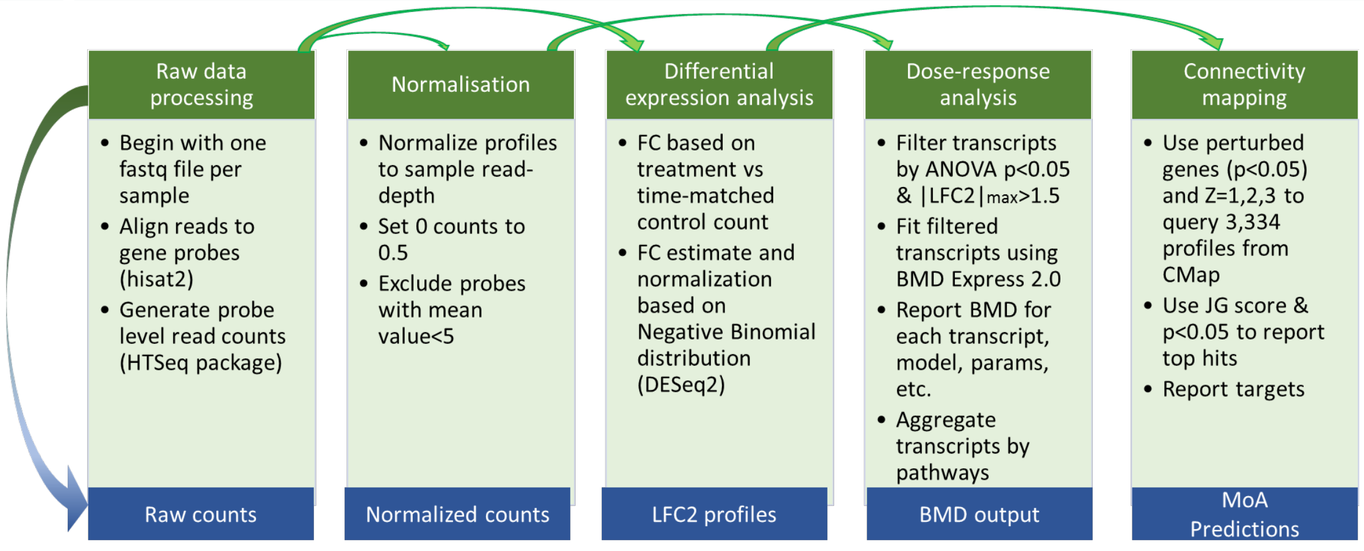


Figure 1. The laboratory workflow includes standardized expansion of MCF7 cells from cryopreserved stocks, automated seeding of cells in 384 well plates, acoustic dispensing of test chemicals in an uniquely randomized pattern on each test plates and either generation of cell lysates for TempO-Seq analysis (Track 1) or labeling of cells with CellEvent Caspase 3/7 and propidium iodide for a high content-imaging based apoptosis / cell viability assay (Track 2).

3 HTTr Data Analysis Pipeline



4 HTTr Assay Performance

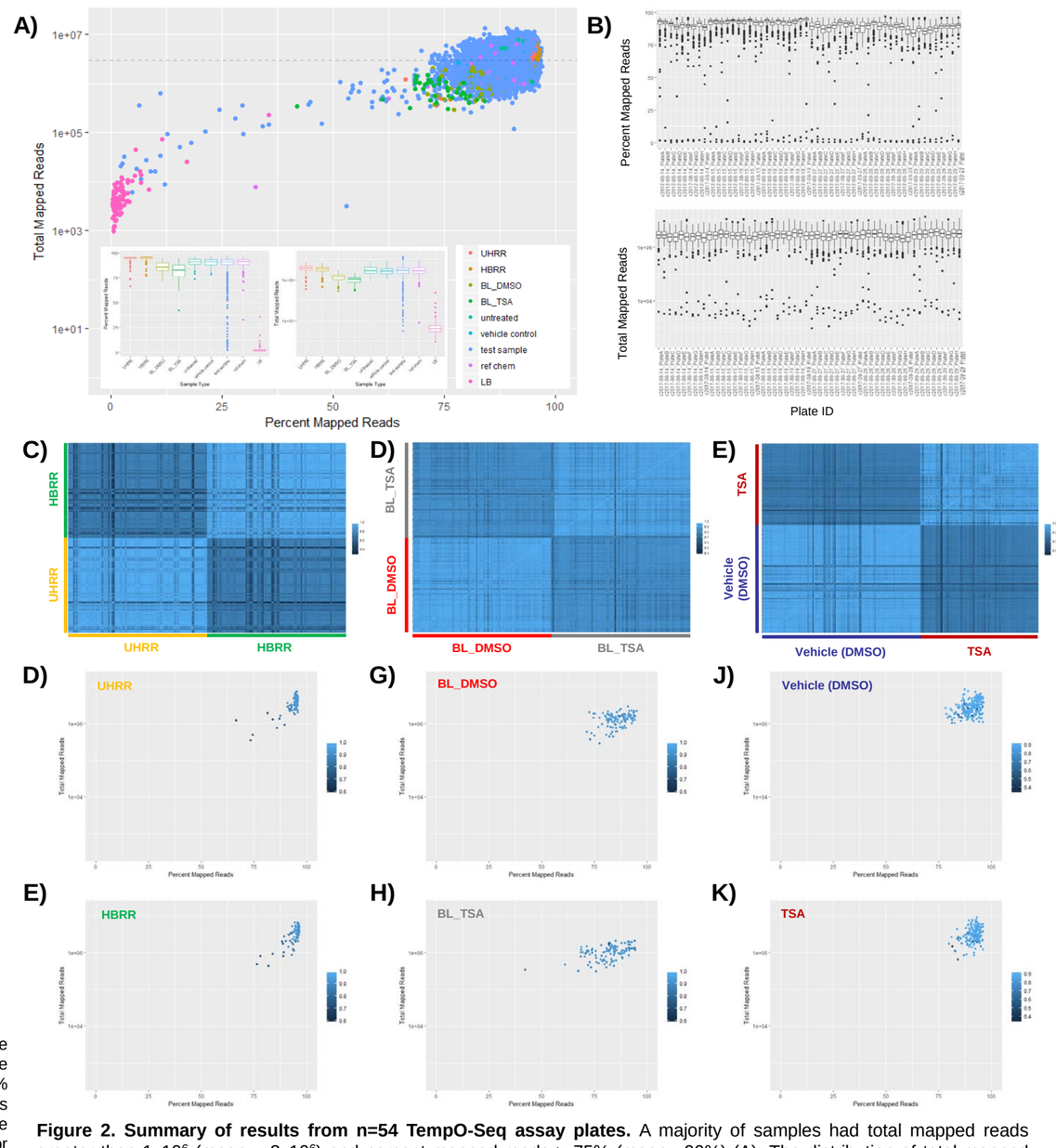


Figure 2. Summary of results from n=54 TempO-Seq assay plates. A majority of samples had total mapped reads greater than $1x10^6$ (mean ~ $3x10^6$) and percent mapped reads > 75% (mean ~90%) (A). The distribution of total mapped reads and percent mapped reads were consistent across assay plates (B). Correlations of like QC samples across plates was high (>0.9) in most cases. Paired QC samples (i.e. UHRR vs. HBRR, BL_DMSO vs BL_TSA, DMSO vs TSA) showed distinct differences in the strength of correlations (C-D). Poor correlations with like samples observed in a minority of QC samples had no apparent relationship with total mapped reads or percent mapped reads (D-K).

5 POD Determination BMDExpress2.0

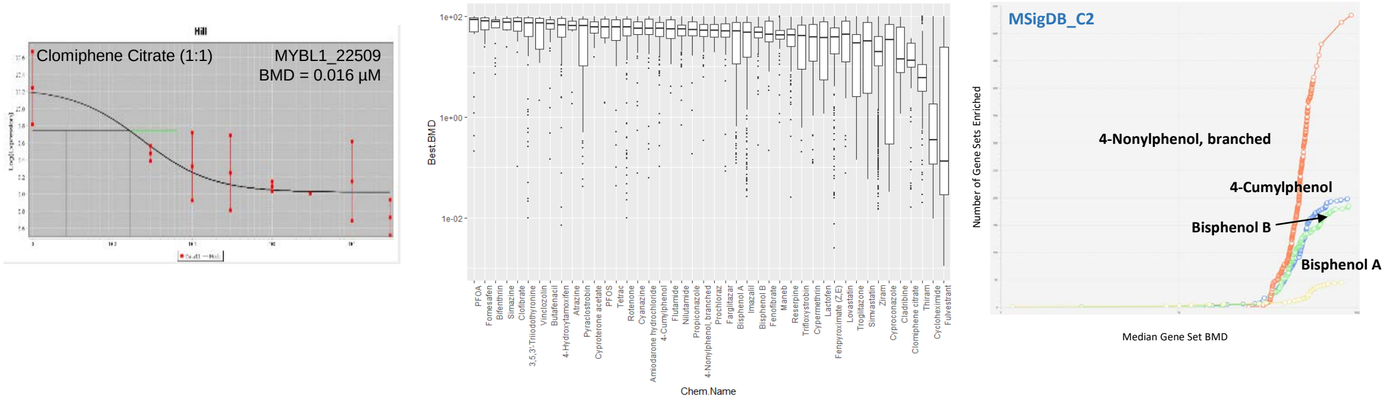


Figure 3. Benchmark Dose Modeling with BMDExpress2.0. The BMDExpress2.0 transcriptomic concentration-response modeling tool was used to fit concentration-response curves for the respective test chemicals to each probe in the TempO-Seq whole transcriptome assay. (A) An example concentration-response curve for an estrogen-responsive gene (MYBL1) following treatment of MCF7 cells with clomiphene citrate, an estrogen receptor antagonist. Benchmark doses were defined as concentrations which produce a >10% change in a response. (B) The distribution of probe level BMDs for 44 example chemicals. Heterogeneity in the potency and distribution of probe-level BMDs were observed across chemicals. (C) Probes with significant concentration-responses were input into a gene set enrichment analysis using the Molecular Signatures C2 curated gene set database. The accumulation plot illustrates the number of enriched gene sets (y-axis) and the respective probe-level median BMD within each gene set (x-axis) for a group of estrogen receptor agonists. Difference in potency were observed at the pathway level.

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