EPA
United States
Environmental Protection
Agency

High Throughput In Vitro Assays for Chemical Safety Evaluation

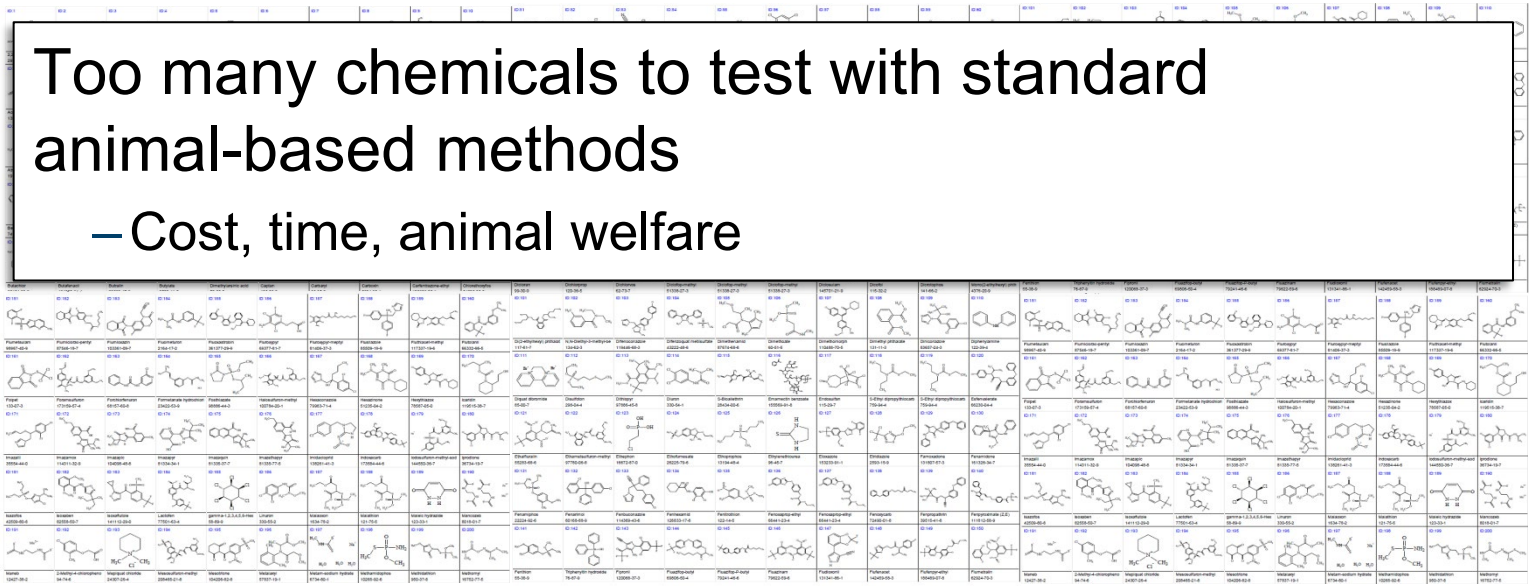
Richard Judson
Center for Computational Toxicology and Exposure
Office of Research and Development

Advanced Cell Culture & Tissue Engineering lab course (BIOC4201)
at Carleton University
March 4, 2021

Problem Statement

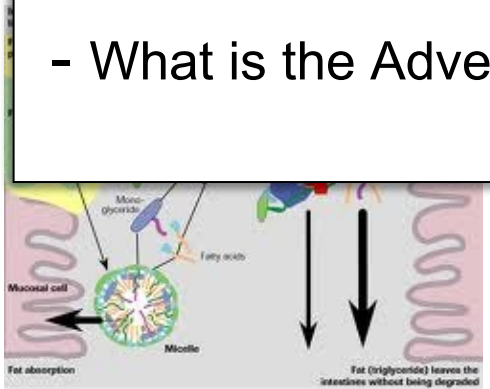
Too many chemicals to test with standard animal-based methods

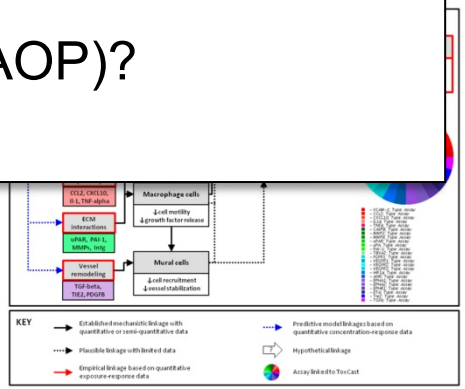
- Cost, time, animal welfare



Need for better mechanistic data

- Determine human relevance
- What is the Adverse Outcome Pathway (AOP)?





EPA United States Environmental Protection Agency

Computational Toxicology

- Identify biological pathways of toxicity (AOPs)
- Develop high-throughput *in vitro* assays to test chemicals
- Test “Human Exposure Universe” chemicals in the assays
- Develop models that link *in vitro* to *in vivo* hazard
- Use pharmacokinetic models to predict activating doses
- Develop exposure models for all chemicals
- Add uncertainty estimates
- Create high-throughput risk assessments

High-throughput Methods

- High-throughput Transcriptomics (HTTr)
  - Measure changes in levels of all expressed genes
  - Targeted RNASeq
- High-throughput Phenotypic Profiling (HTPP)
  - Measures changes in cell compartment size, shape, texture
  - Cell Painting
- Target-specific High-throughput Screening Assays (HTS)
  - Multiple technologies to measure specific chemical-target interactions
- *Determine Mechanisms of Action and Potency*

Experimental Workflow

Cryopreserved Cell Stocks

Cell Expansion

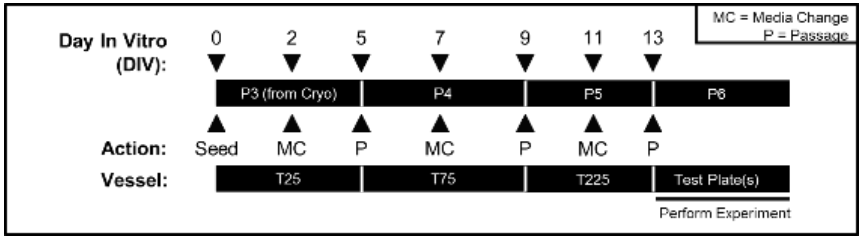
Cell Plating

BioTek MultiFlo ™ FX

Dispensing Test Chemicals

LabCyte Echo® 550 Liquid Handler

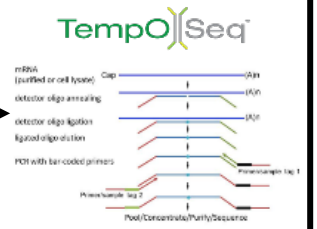
Standardized Expansion Protocol



Track 1: Targeted RNA-Seq

Generating Cell Lysates

TempO-Seq WT



Track 2: Apoptosis / Cell Viability

Reagent Dispensing

High Content Imaging

Perkin Elmer Opera Phenix™ High Content Screening System

HTPP with the Cell Painting Assay

Cell Painting is a profiling method that measures a large variety of phenotypic features in fluoroprobe labeled cells *in vitro*.

- High-throughput
- Scalable
- Amenable to lab automation
- Deployable across multiple human-derived cell types.
- Reproducible
- Cost-effective (¢ / well)
- Infrastructure investment
- High volume data management

Laboratory & bioinformatics workflows for conduct of this assay have been established at CCTE.

OPEN ACCESS Freely available online PLOS ONE

Multiplex Cytological Profiling Assay to Measure Diverse Cellular States

Sigrun M. Gustafsdottir, Vebjorn Ljosa, Katherine L. Sokolnicki, J. Anthony Wilson, Deepika Walpita, Melissa M. Kemp, Kathleen Petri Seiler, Hyman A. Carrel, Todd R. Golub, Stuart L. Schreiber, Paul A. Clemons, Anne E. Carpenter, Alykhan F. Shamji

Broad Institute of Harvard and MIT, Cambridge, Massachusetts, United States of America

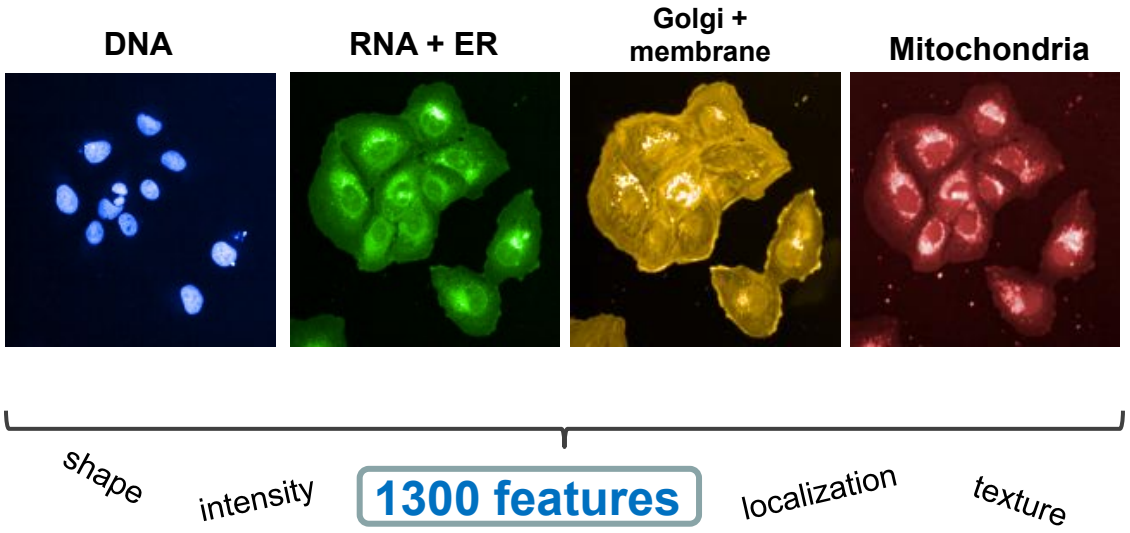
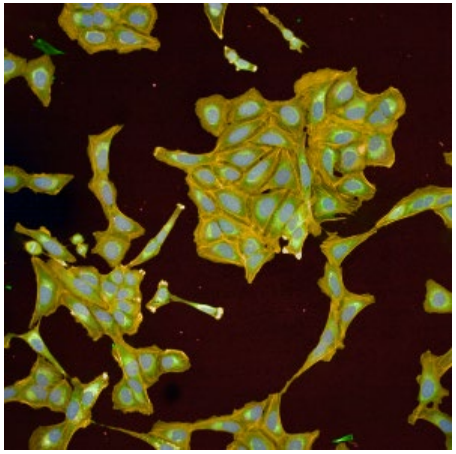
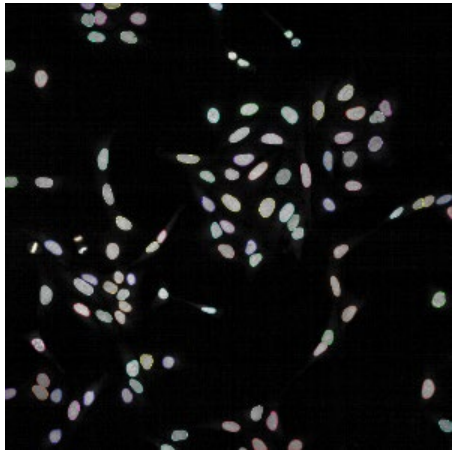
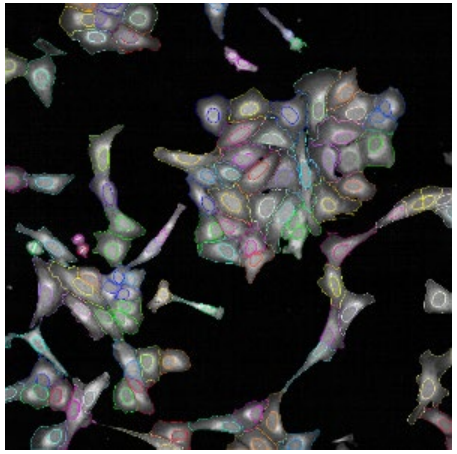
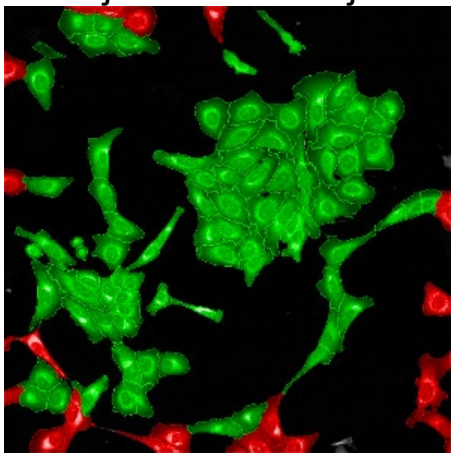
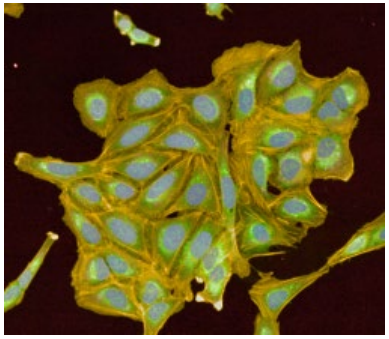
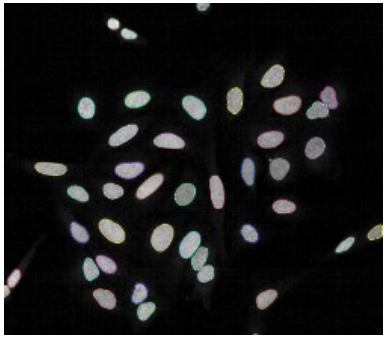
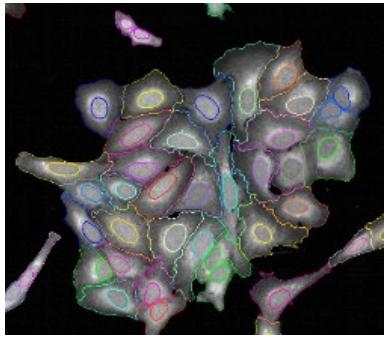


Image Analysis Workflow → Image Segmentation

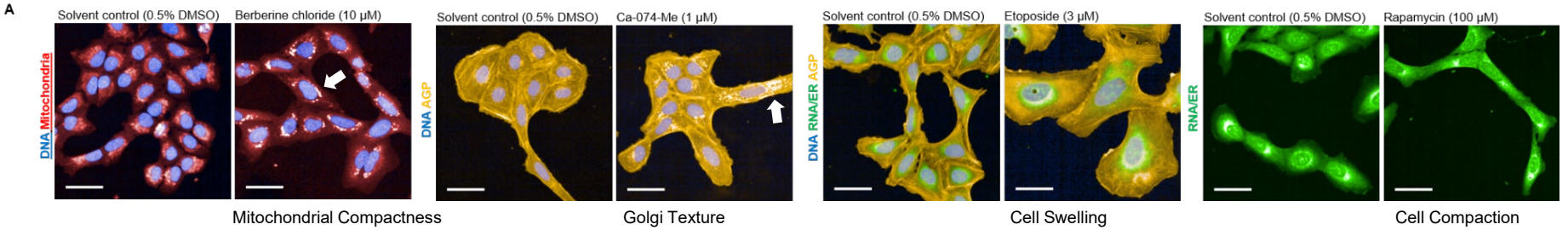
1. find nuclei

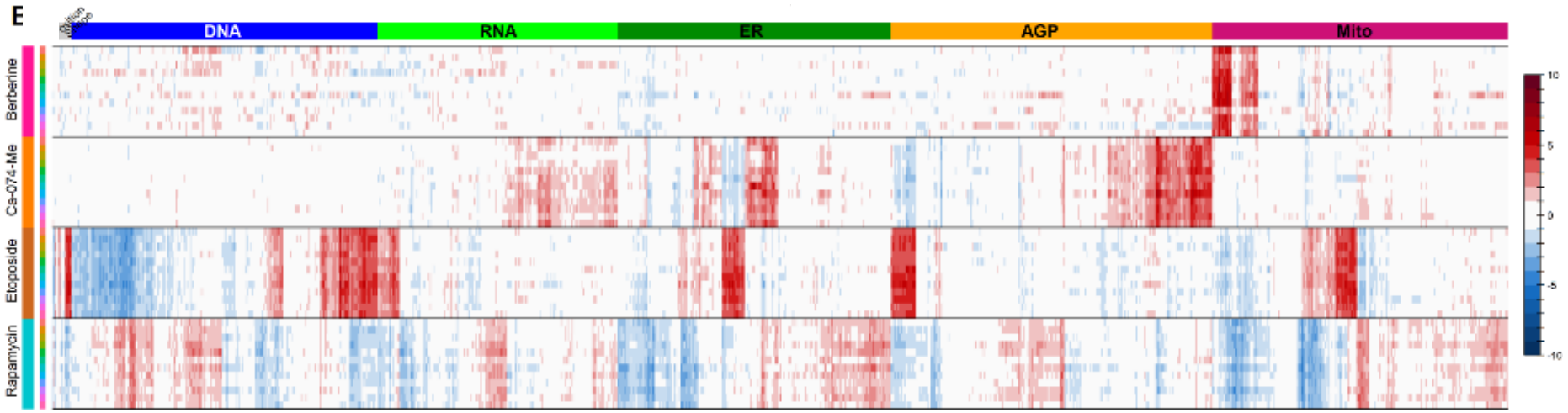
2. find cell outline

3. reject border objects

Examples of Chemical Induced Phenotypes

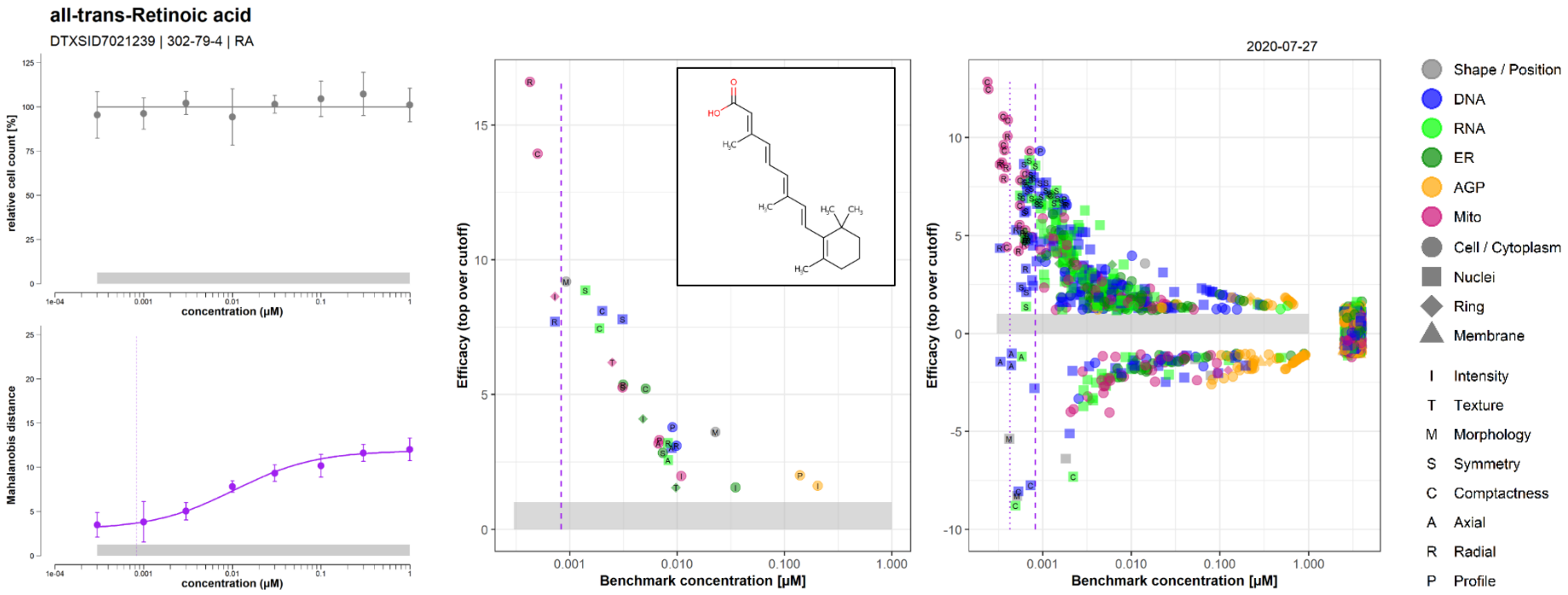




- Strong phenotypes are observed qualitatively and produce distinct profiles when measured quantitatively.

Adapted from Nyffeler et al. Toxicol Appl Pharmacol. 2020 Jan 15;389:114876

Concentration-Response Modeling Example

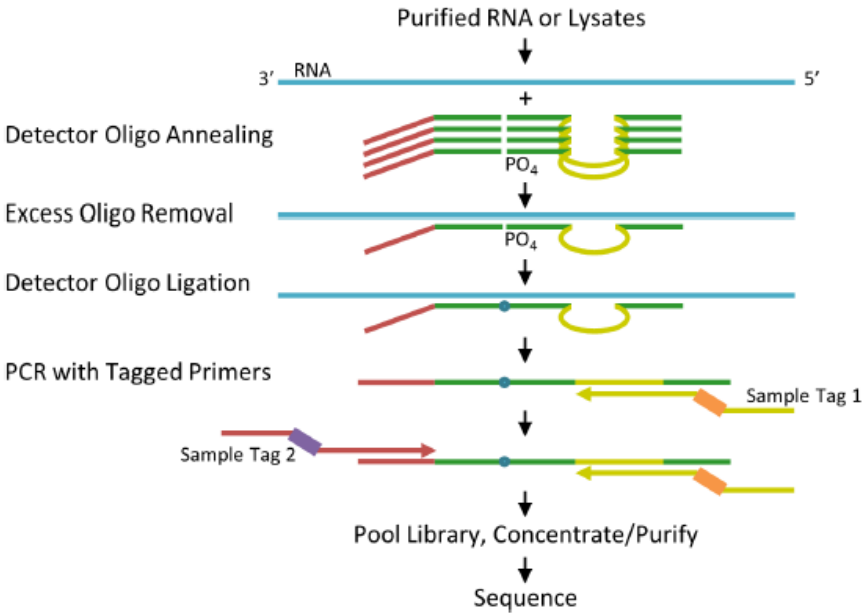


- At each concentration score each of 1300 features
- Do concentration-response analyses to get potency estimate
- Consolidate features into 49 categories for better interpretation

HTTr Using TempO-Seq Platform

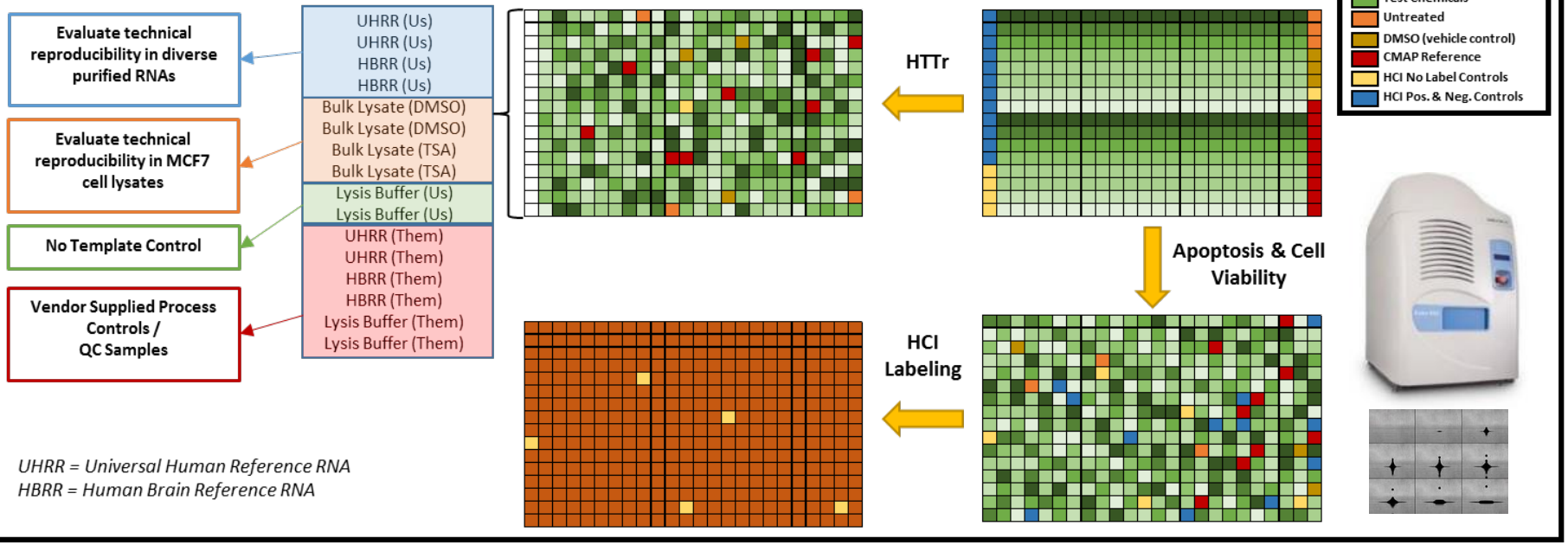
- The **TempO-Seq** human whole transcriptome assay measures the expression of ~21,100 transcripts.
- Requires only picogram amounts of total RNA per sample.
- Compatible with purified RNA samples or **cell lysates**.
- Transcripts in cell lysates generated in 384-well format barcoded to well position
- Scalable, targeted assay:
  - Measures transcripts of interest
  - Greater throughput and requires lower read depth than RNA-Seq
  - Ability to attenuate highly expressed genes

TempO-Seq Assay Illustration



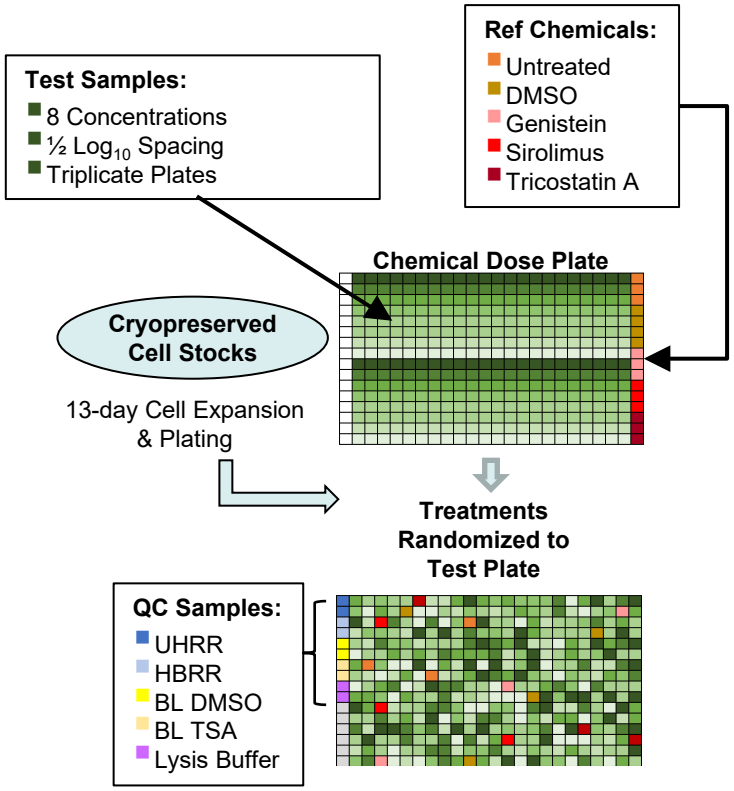
Treatment Randomization & Quality Control Samples

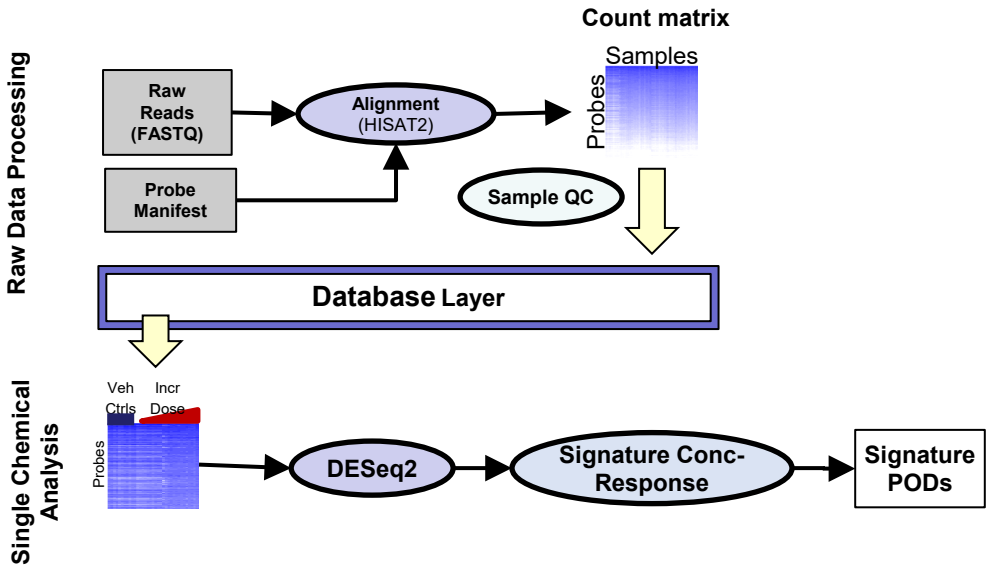
Treatment Randomization: *Each test plate uniquely randomized with respect to treatment.*
QC Samples: *Quality Control samples included on each plate*



UHRR = Universal Human Reference RNA
HBRR = Human Brain Reference RNA

HTTr Overall Process



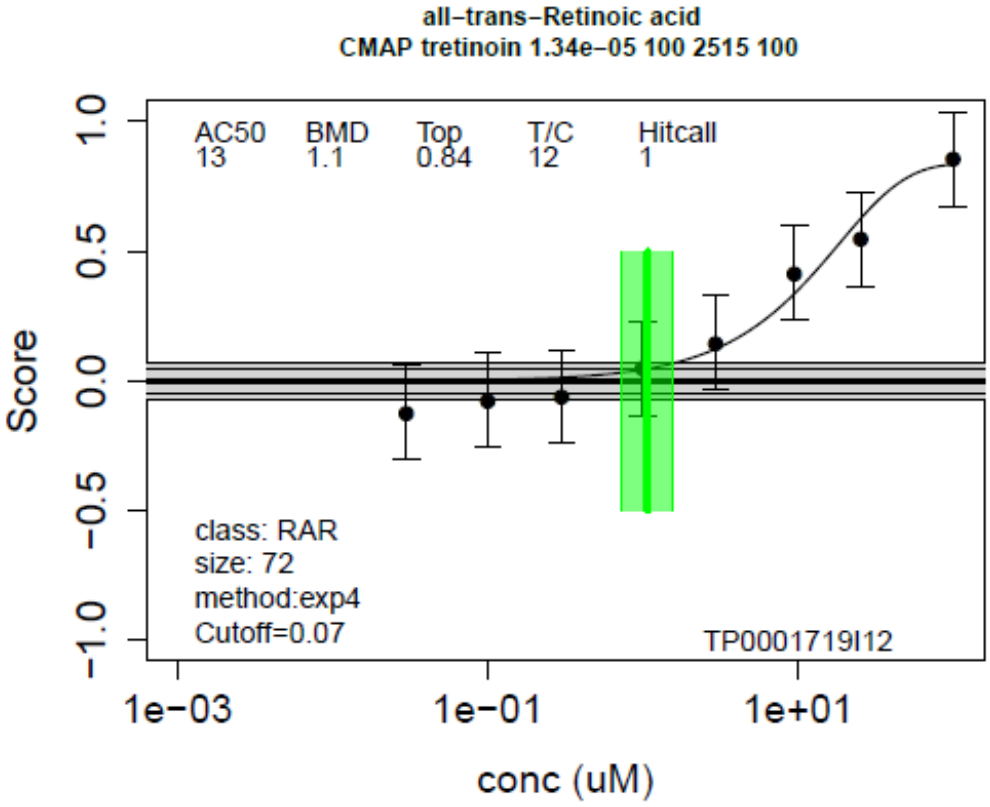


Gene Sets: “Signatures”

- Understanding the results of changes in expression of 10,000-20,000 genes is hard
- Group genes into gene sets (“Signatures”)
- Examples of signature types
  - Genes that are perturbed in diseased tissue vs. health tissue
  - Genes perturbed in individuals with congenital diseases vs. those without
  - Genes perturbed by drugs or other chemicals
  - Genes perturbed by gene knockdowns / knockouts
- Example use
  - If a chemical perturbs the genes upregulated in a cancer type, the chemical is a candidate carcinogen (or candidate anti-cancer drug)

- Each signature has a hand-annotated “super target” class to help with annotation
- ~10,000 signatures
- ~1000 super targets

Example Signature Concentration-Response plot



Confidence Interval (CI) around points from the fitting error term

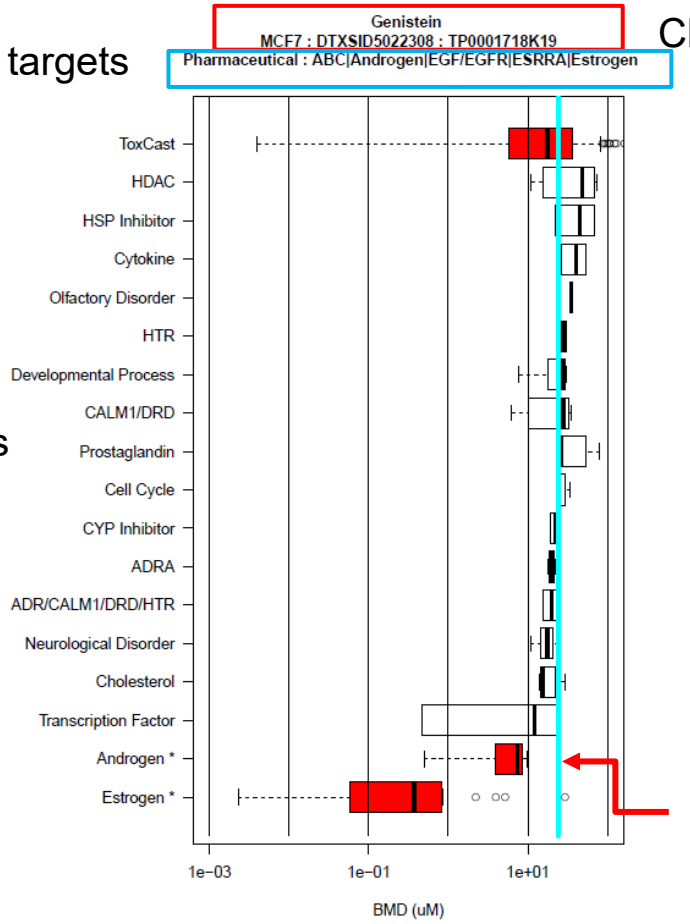
Outer gray band is 95% CI of null dist. Inner lines are benchmark response

Green vertical band is BMD and 95% CI

Super Target Summary Plot

Use class and known targets

Chemical name, DTXSID and sample ID



Super targets

Boxplot shows range of BMD values for signatures for the super targets

Red indicates that the super target is a target of the chemical

Median of all super target BMD medians

Summary

- Need to Screen thousands of chemicals for potency and mechanism of action
- We can now do this with HTPP, HTTr and HTS
- Application areas in current use
  - Prioritizing chemicals for further investigation
  - Clustering chemicals by activity profile
  - Identifying areas of concern for emerging contaminants
  - Estimating safe exposure levels for chemicals
  - Animal-free evaluation of chemical safety for cosmetics ingredients (with Unilever)

Acknowledgements

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Hazard Identification Workflow

- A flexible, portable and cost-efficient platform to comprehensively evaluate the potential biological pathways and processes impacted by chemical exposure
  → High-throughput transcriptomics (HTTr)
- Identify the concentration at which biological pathways / processes begin to be impacted
- Assign putative biological targets for chemicals

A strategic vision and operational road map for computational toxicology at the U.S. Environmental Protection Agency