

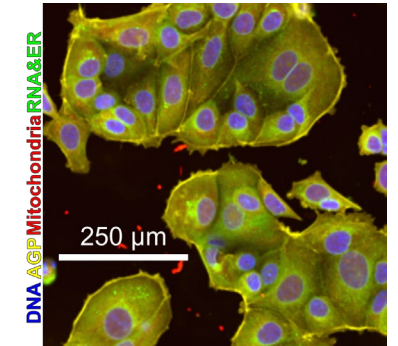
# Incorporating Metabolic Competence into High-Throughput Profiling Assays

Amanda Jurgelewicz<sup>1,2</sup>, Kristen Breaux<sup>1</sup>, Clinton Willis<sup>1</sup>, Felix R. Harris<sup>1,2</sup>, Gabrielle Byrd<sup>1,2</sup>, Joshua Witten<sup>1,2</sup>, Derik Haggard<sup>1</sup>, Joseph Bundy<sup>1</sup>, Logan Everett<sup>1</sup>, Chad Deisenroth<sup>1</sup> & Joshua A. Harrill<sup>1</sup>  
1. CCTE, US EPA, Durham, NC 2. ORISE, Oak Ridge, TN

The US EPA has proposed high-throughput profiling (HTP) assays as a first-tier approach for chemical hazard characterization

## High-Throughput Phenotypic Profiling (HTPP) with Cell Painting

Detecting changes in cell morphology



VM7Luc4E2 cells (0.1% DMSO)

Complementary assays for assessing chemical bioactivity

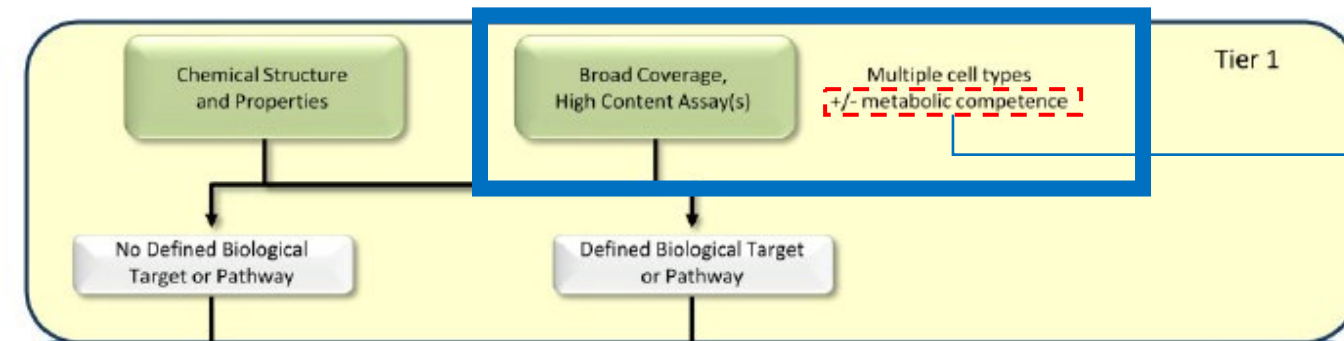


Cell lysates are shipped to BioSpyder for sequencing

## High-Throughput Transcriptomics (HTTr) with TempO-Seq

Detecting changes in gene expression

Thomas, et al, 2019 (PMID: 30835285)



One limitation of using new approach methods (NAMs) for chemical risk assessment is the lack of metabolic competence

## Alginate Immobilization of Metabolic Enzymes (AIME)

Hopperstad, et al, 2022 (PMID: 35172002)



$\text{dH}_2\text{O} + \text{Alginate} \rightarrow \text{parent chemicals}$

Cells are treated with AIME conditioned media

Hepatic S9 + Alginate  $\rightarrow$  metabolites

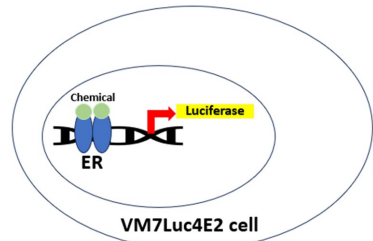
AIME is a platform for incorporating in vitro metabolism into high-throughput assays

# Incorporating Metabolic Competence into High-Throughput Profiling Assays

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## Proof-of-Concept Experiment in VM7Luc4E2 (breast) Cells:

### Estrogen Receptor Transactivation (ERTA)



Biotransformation assay control



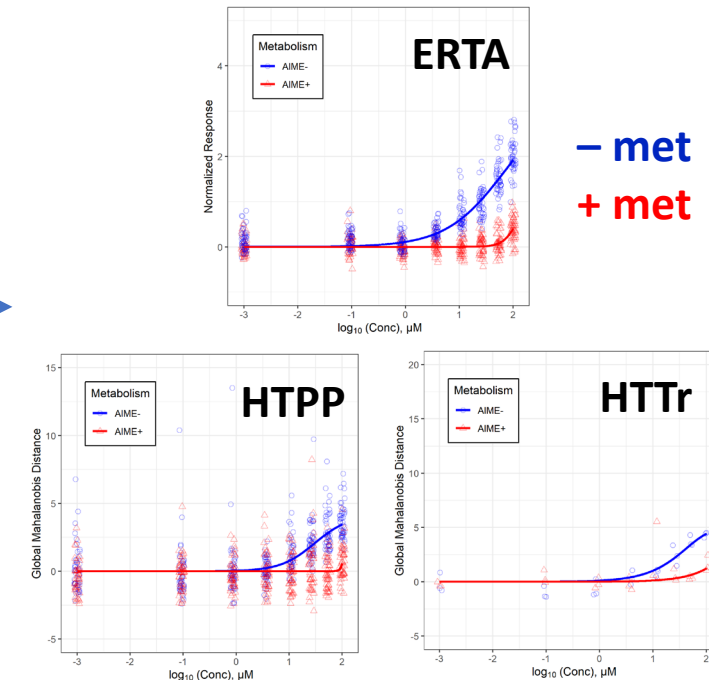
### High-Throughput Profiling Assays

HTPP and HTTr

3 chemicals tested in 3 assays:

Estrogenic Chemical	Expected AIME Shift in ERTA
Ethylparaben	Inactivation
trans-Stilbene	More Activation
Daidzein	No Change

Preliminary Results – Do not Cite or Quote



Applying the workflow developed in this experiment to more cell lines and larger chemical sets will help address important limitations and uncertainties for using NAMs-based methods for chemical risk assessment.

Overall activity aligned with expectations for all 3 chemicals

For more information, contact:  
Amanda Jurgelewicz  
([Jurgelewicz.Amanda@epa.gov](mailto:Jurgelewicz.Amanda@epa.gov))