

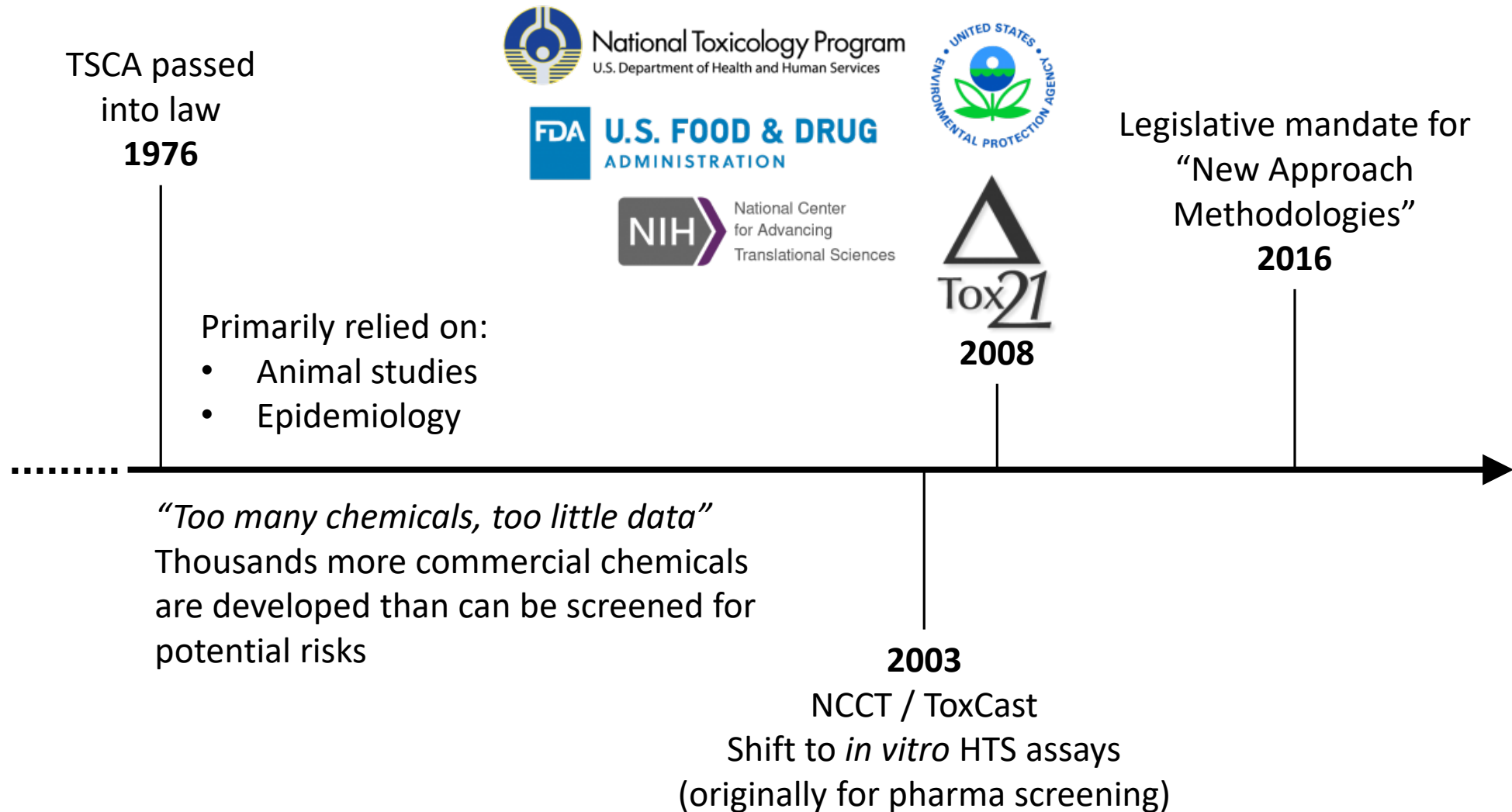
High-throughput Transcriptomics for Accelerated Toxicity Screening

Logan J. Everett, Ph.D.



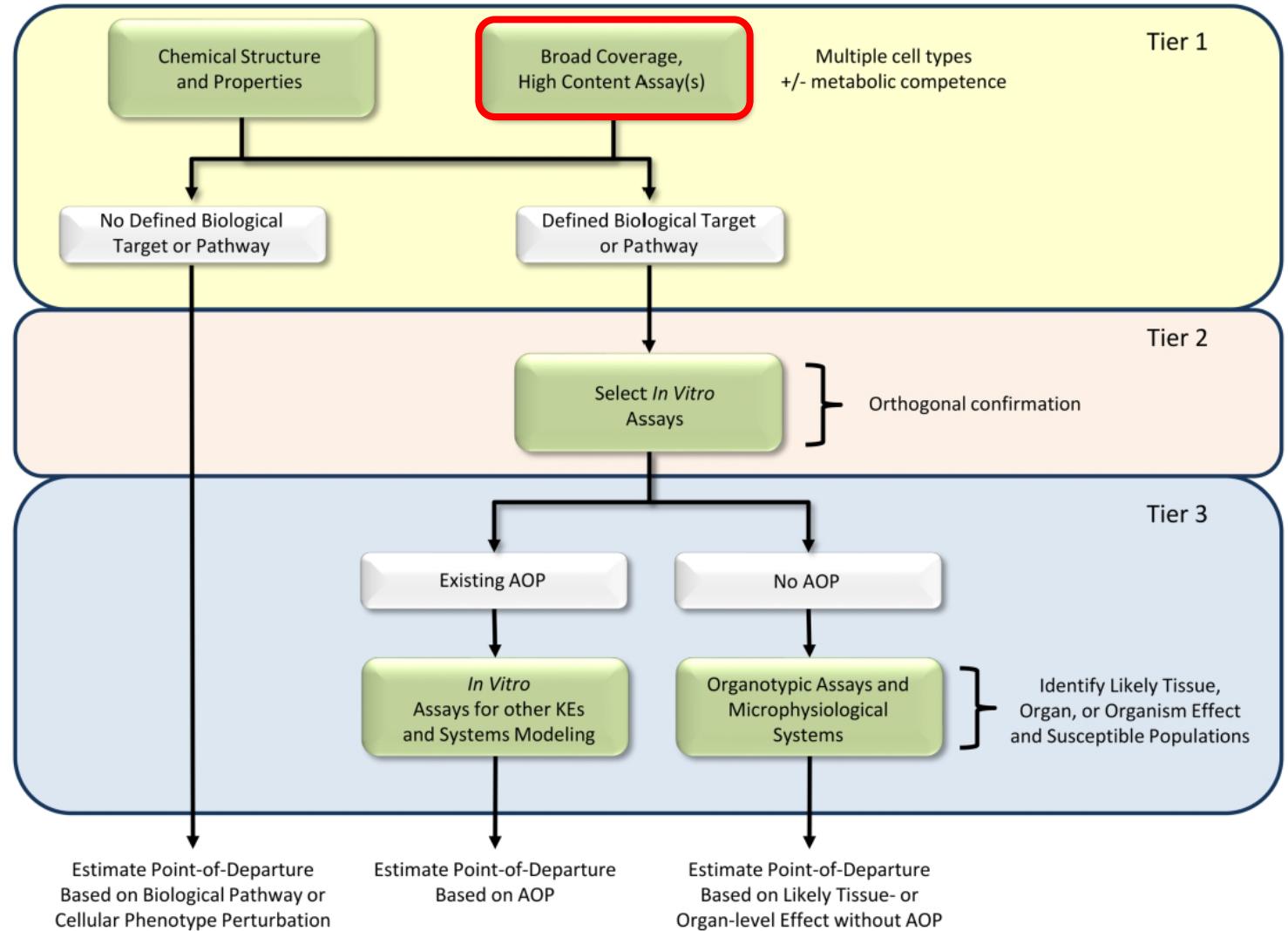
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those of the presenter and do not necessarily
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U.S. Environmental Protection Agency*

History of Chemical Risk Assessment



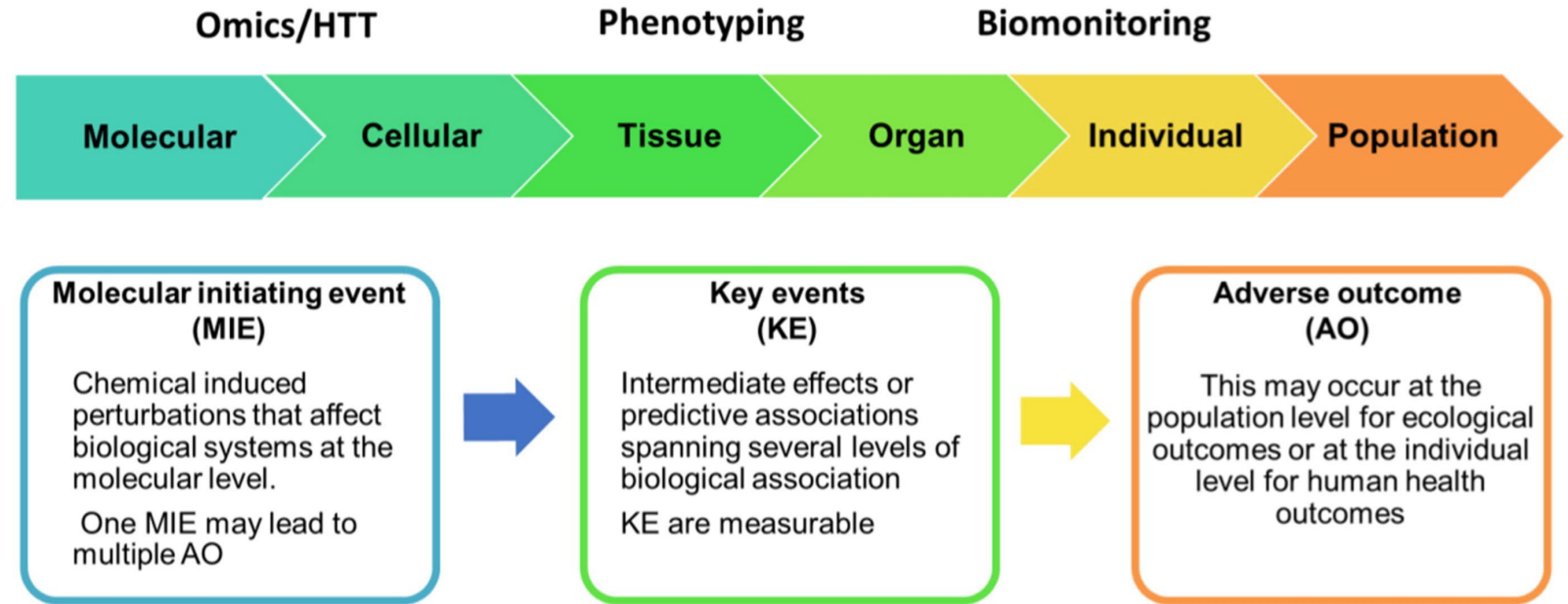
Tiered Testing Strategy

- Use transcriptomics to:
 - Screen for bioactive chemicals
 - Predict benchmark dose
 - Infer mode of action
 - Predict adverse outcomes
- Adverse Outcome Pathways (AOPs) link cell & molecular perturbations to organism-level effect



Overview of AOP Framework

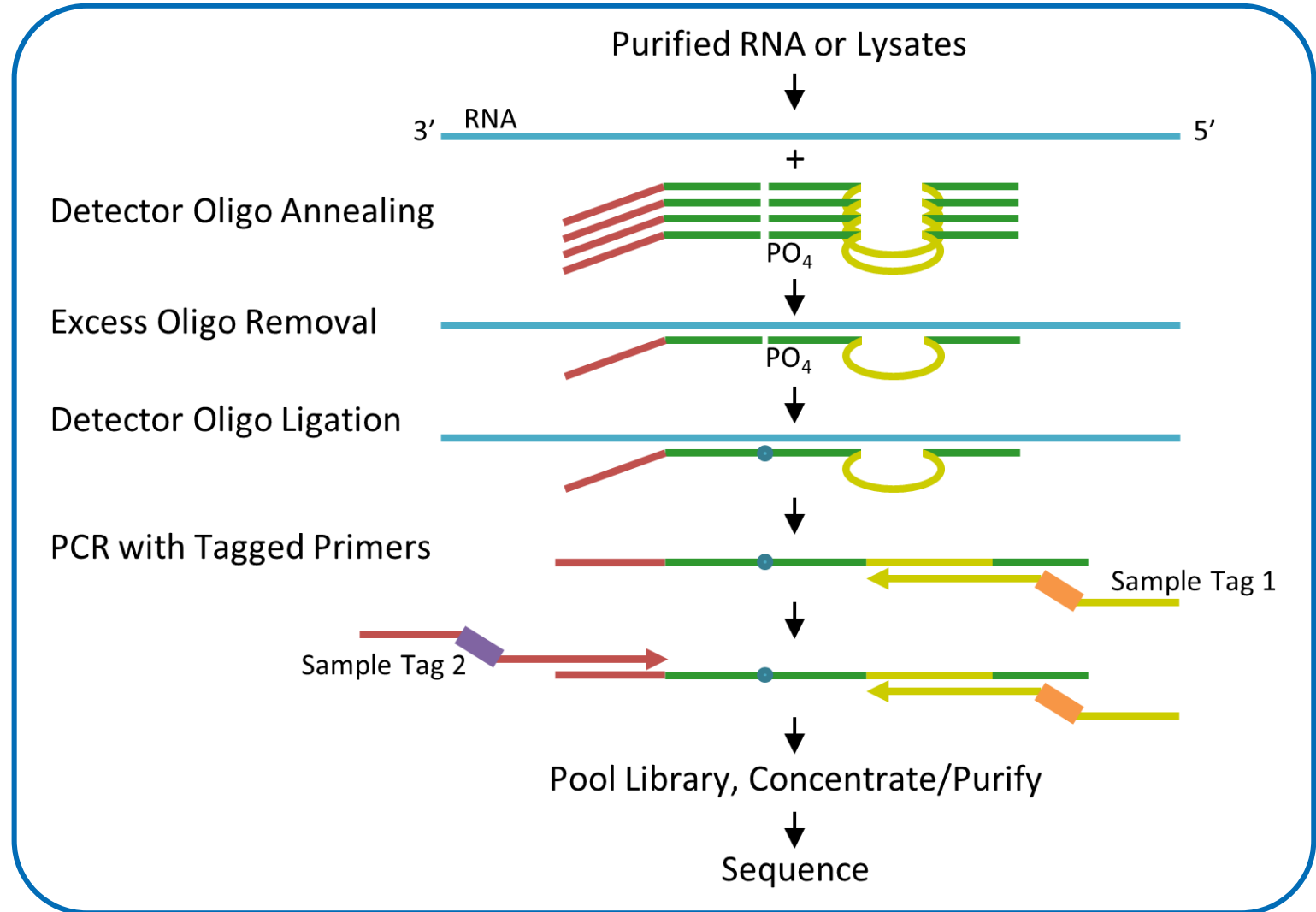
- Use transcriptomics to:
 - Screen for bioactive chemicals
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- **Adverse Outcome Pathways (AOPs)**
link cell & molecular perturbations to
organism-level effect



Hamm, et al. Toxicol in Vitro 2017

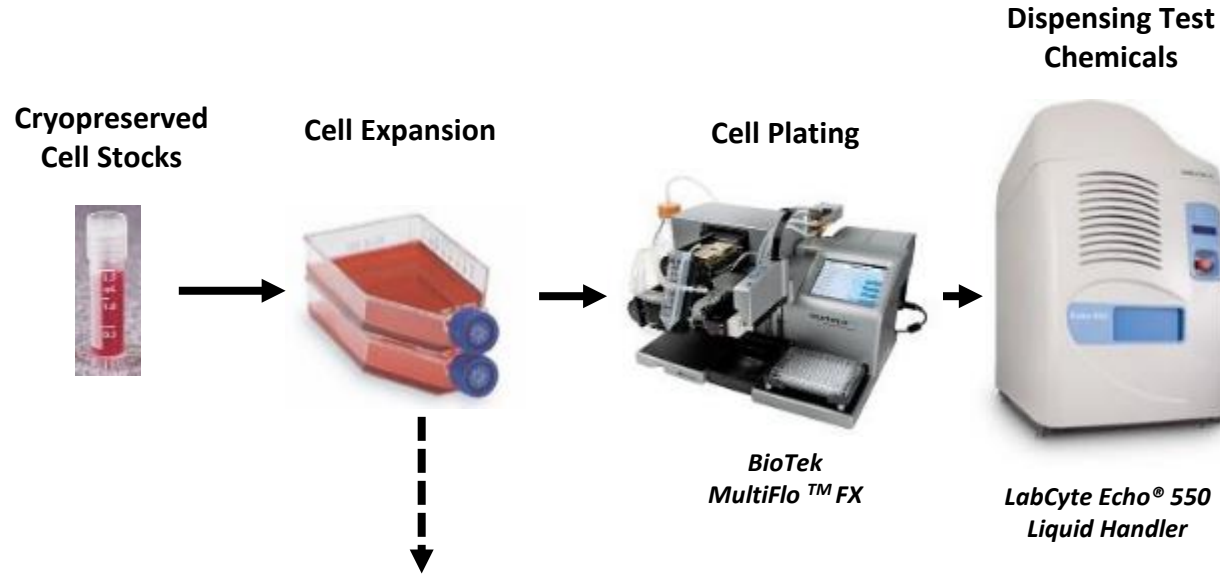
TempO-seq Enables High-throughput Transcriptomics

- Use purified RNA or cell lysates
- Probe set for whole human transcriptome target ~21,000 human genes
- Captures majority of signal with much lower sequencing depth (~6M reads)
- Attenuation of highly expressed genes reduces depth further (~3M reads)
- Barcoding and pooling allows hundreds of samples per flowcell
- Data pre-processing is much faster

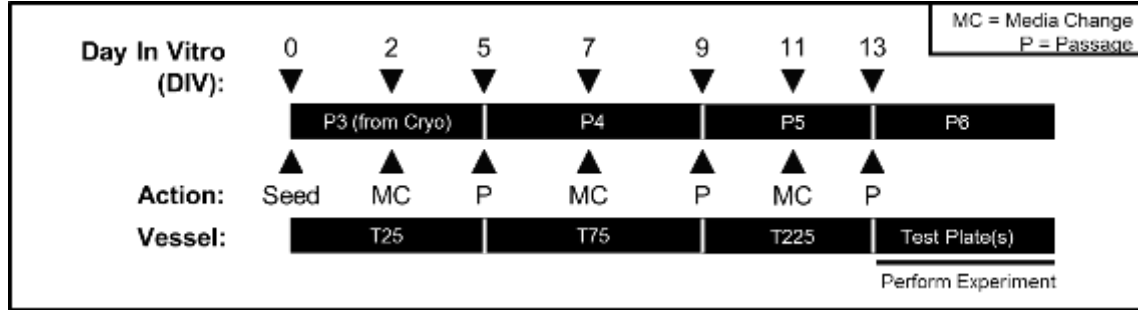


Yeakley, et al. PLoS ONE 2017

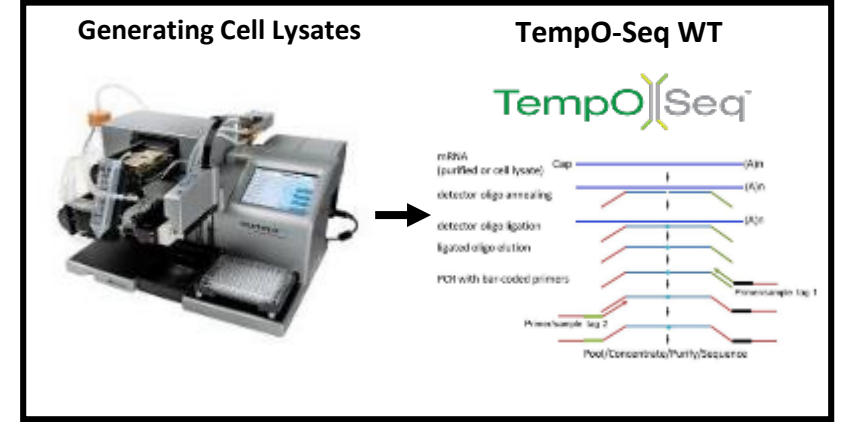
Screening Protocol



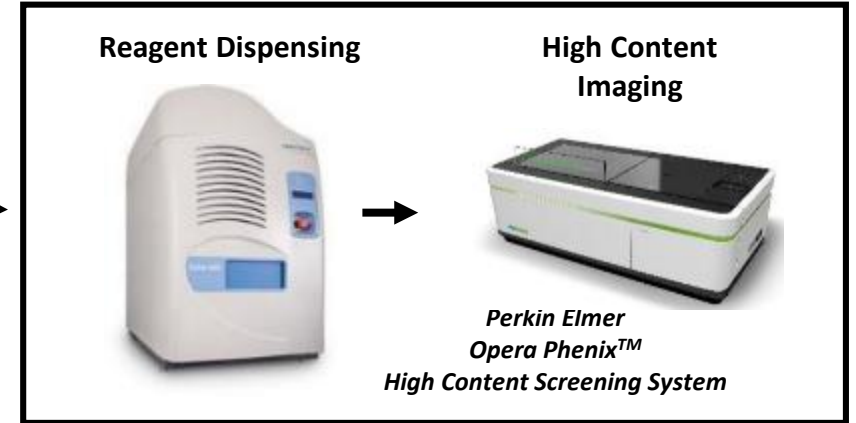
Standardized Expansion Protocol



Track 1: Targeted RNA-Seq

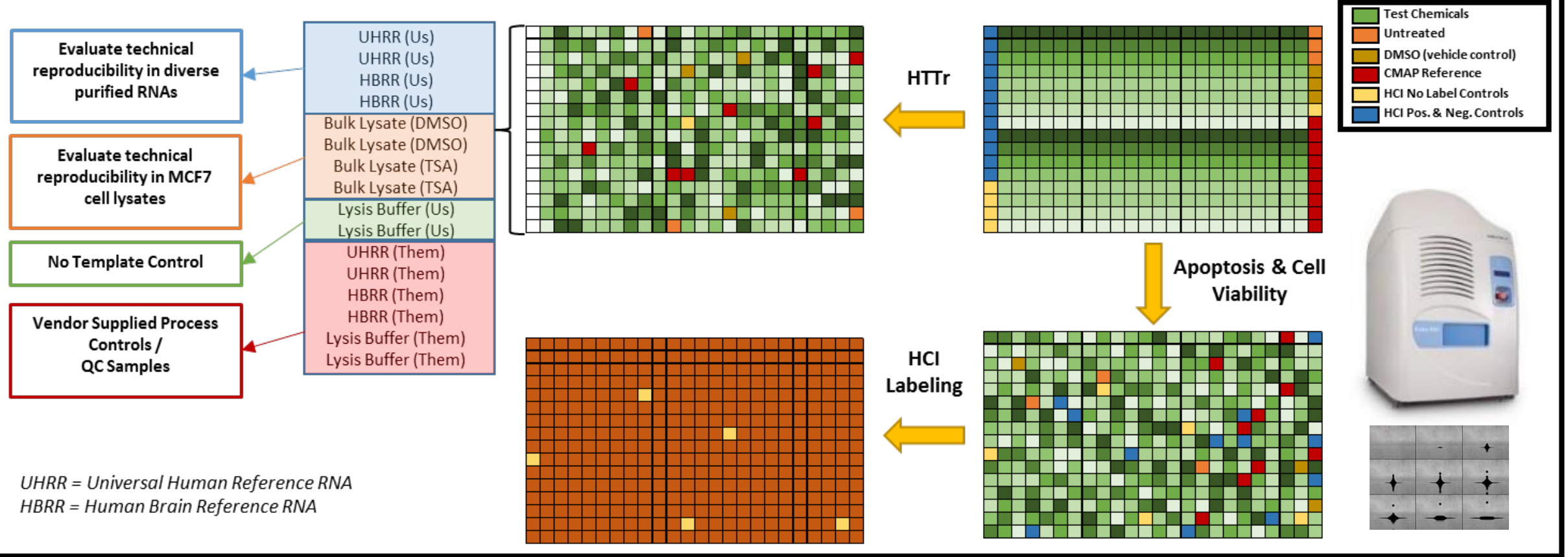


Track 2: Apoptosis / Cell Viability



Screening Protocol

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



First Screen: MCF-7 Cells

Study 1: Pilot Screen
6,804 Samples

Study 2: Large Scale Screen
~54,432 Samples (>50TB)

Parameter	Multiplier	Notes	Multiplier	Notes
Cell Type(s)	1	MCF-7	1	MCF-7
Culture Condition	2	DMEM + 10% HI-FBS PRF-DMEM + 10% CS-HI-FBS	1	DMEM + 10% HI-FBS ^a
Chemicals	44	Mechanistic Diversity w/ Redundancy	2,112	ToxCast ph1, ph2, e1k / ph3
Time Points:	3	6, 12, 24 hours	1	6 hours
Assay Formats:	2	TempO-Seq HCI Cell Viability & Apoptosis	2	TempO-Seq HCI Cell Viability & Apoptosis
Concentrations:	8	3.5 log ₁₀ units; semi log ₁₀ spacing	8	3.5 log ₁₀ units; semi log ₁₀ spacing
Biological Replicates:	3	--	3	--

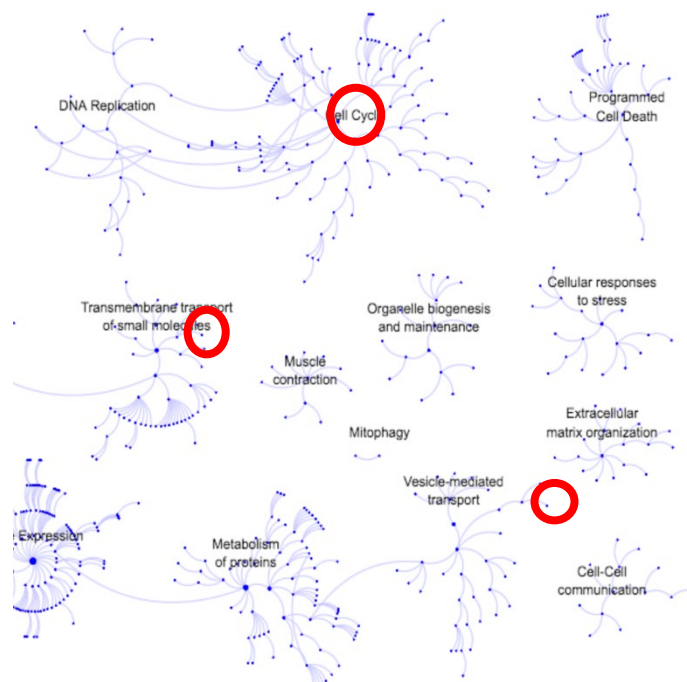
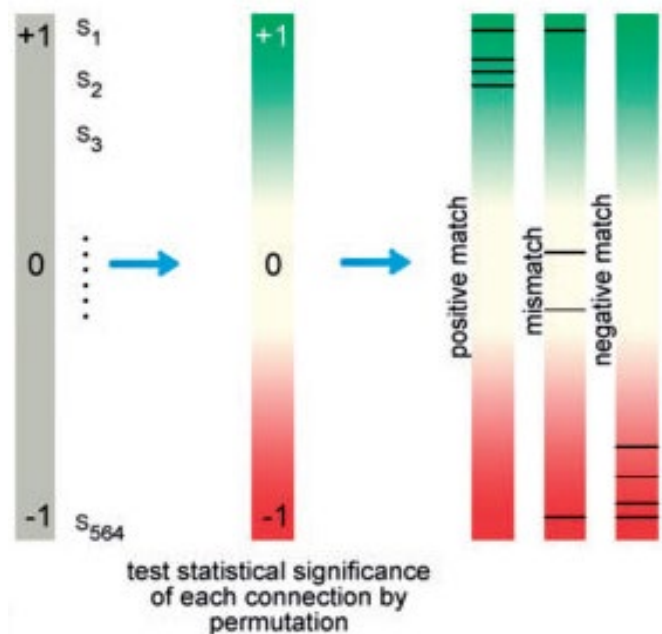
Next Screens: HepaRG (~2k chemicals) + additional cell type

Molecular Target Prediction

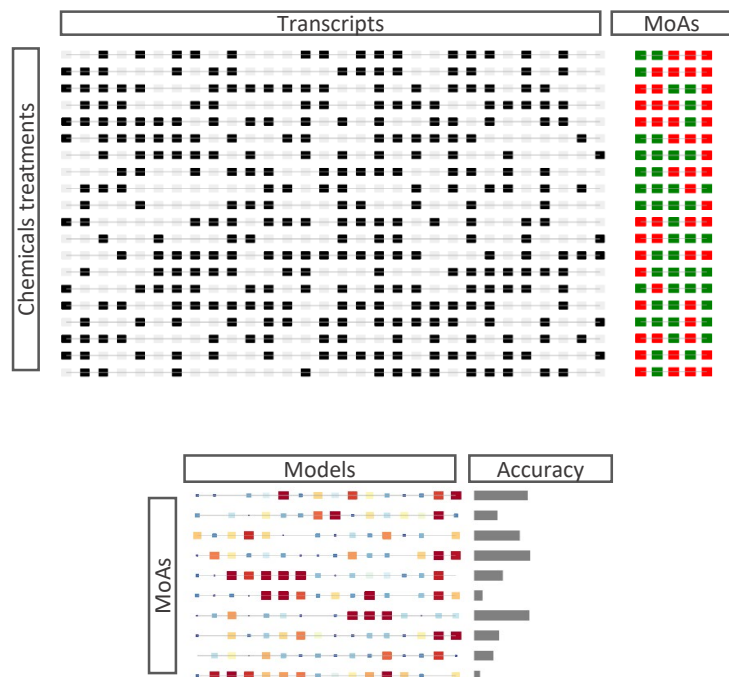
Connectivity mapping
analysis using DEGs and CRGs

Pathway / Network analysis
using DEGs and CRGs

Machine learning to build
Target-specific models

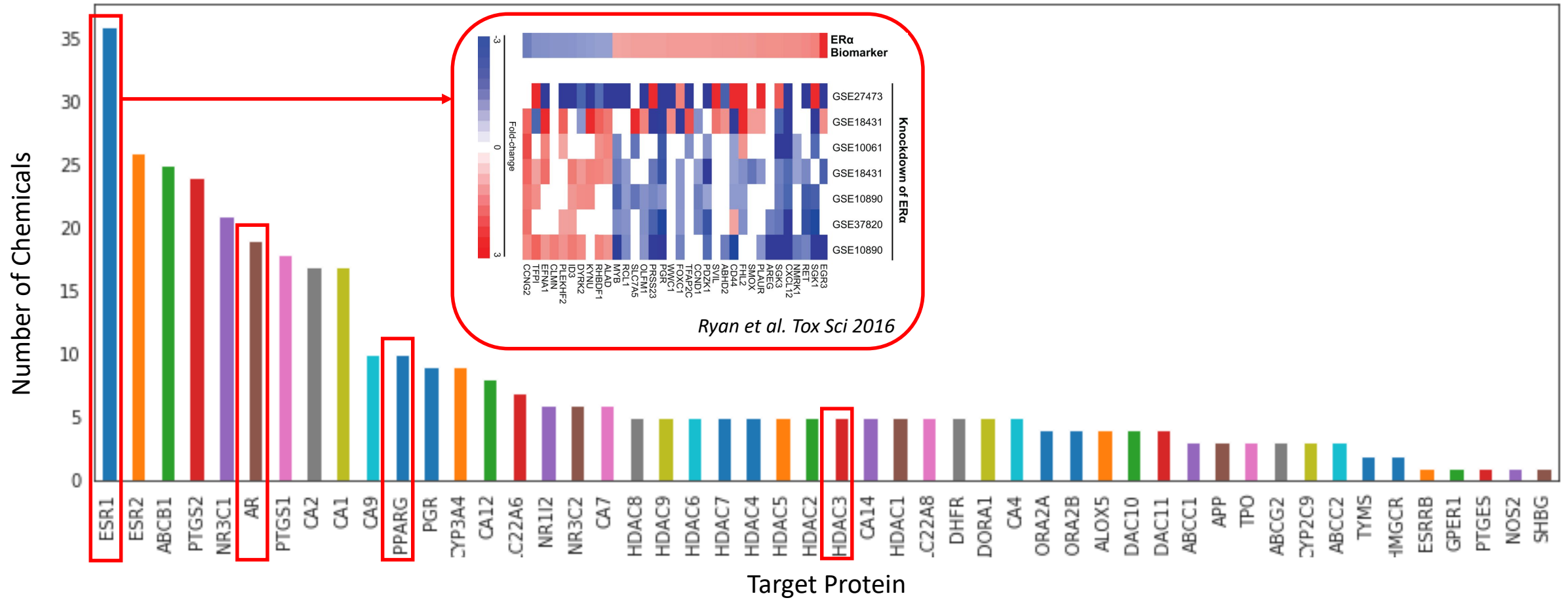


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Chemicals with Known Targets

All Chemicals in Phase-I MCF-7 Screen with Annotated Target



Acknowledgements

Rusty Thomas
Joshua Harrill
Imran Shah
Richard Judson
Woody Setzer

Postdocs:

Derik Haggard
Thomas Sheffield
Johanna Nyffeler



National Center for Computational Toxicology

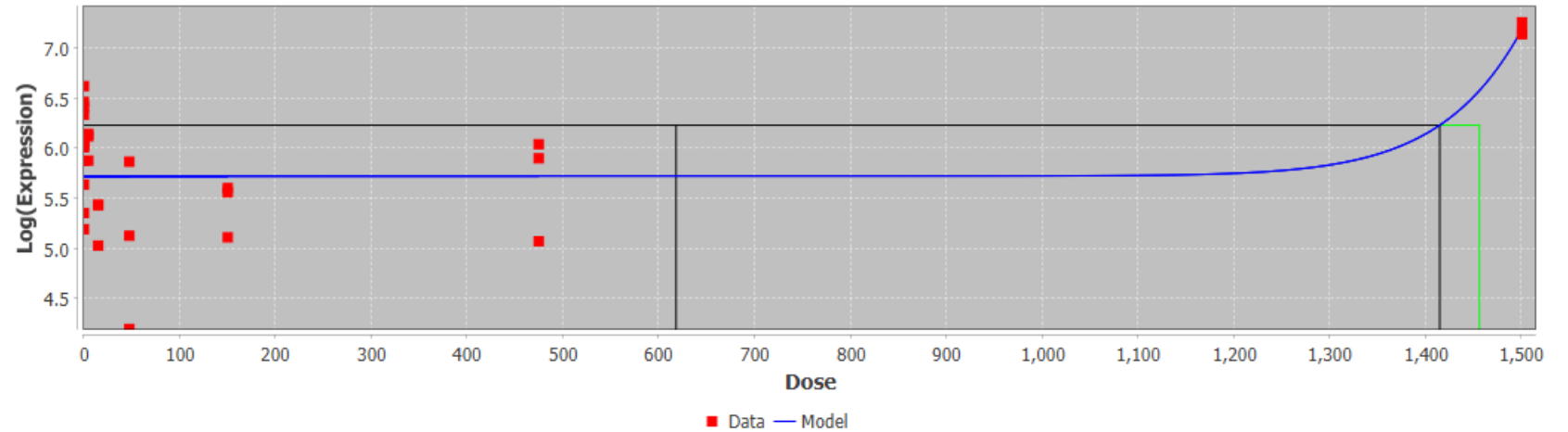




Curve Fitting & POD Estimation

- **Goal:** Determine concentration at which bioactivity occurs
- **Challenge 1:** Statistical properties of seq-based assays violate standard assumptions (normality)
- **Challenge 2:** Summarization of curves/PODs from thousands of genes
- Use concordance with *in vivo* and HTS results as a guide

BMDExpress – Phillips, et al. Bioinf 2019



ToxCast Pipeline (tcpl) – Filer, et al. Bioinf 2017

